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ANNUAL REPORT

JULY 1, 1975 THROUGH JUNE 30, 1976

PART I



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NATIONAL INSTITUTE OF NEUROLOGICAL AND COMMUNICATIVE DISORDERS AND STROKE

ANNUAL REPORT of Program Activities

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ANNUAL REPORT

July 1, 1975 through June 30, 1976

National Institute of Neurological and Communicative Disorders and Stroke
National Institutes of Health

Director's Report

Introduction

As the NINCDS commences its second quarter century, it does so with a new administrative structure and differing demands on its programs. The administrative reorganization and the continued leveling-off of budgets are indicators of the end of an era--an era of beginning, of rapid growth and expansion, and of maturity. In a more poignant way we are reminded of the ending of an era by the deaths during this past year of the Institute's first director and architect, Dr. Pearce Bailey, and of several loyal and long supporters, Drs. Raymond Carhart, Roger Rossiter, Ludwig von Sallmann, and Wilder Penfield. Each in his own way contributed much to the NINCDS and each will be sorely missed.

The Institute formally took cognizance of its 25th anniversary in November 1975 with special meetings of the present National Advisory Council with many former council members and the present Board of Scientific Counselors with a number of former counselors. A symposium followed at which six invited speakers considered future research prospects: Drs. Francis O. Schmitt and Dominick Purpura for the neurosciences; Drs. Fred Plum and Arthur Ward for neurology and neurosurgery; and Drs. Merle Lawrence and Ira Hirsh for the communicative sciences. At the evening dinner, two of the three former Institute directors, Drs. Masland and MacNichol, joined us, and the third, Dr. Bailey, sent us a message. In mid-December the occasion was climaxed by publication of the 3-volume, 25th anniversary work, The Nervous System, which has since become a best seller, reaching a wide and appreciative audience.

Administrative

One year after administrative reorganization into program areas, the new look has not only survived the trials of transition but shaken down into a reasonably smoothly functioning operation. Geographical contiguity of all NINCDS extramural units did not become a reality until February 1976, when the Westwood building group was able to join the others in the Federal building near the NIH campus. The inevitable dislocations occasioned by this consolidation have been succeeded by a remarkably smoothly running operation, a clear tribute to the many dedicated NINCDS staff members who made it possible. The one major problem with reorganization has been the difficulty in the recruitment of the additional key staff members needed to man the various branches and subprograms. In the FY 1976 appropriation, the Congress provided 32 additional positions, so that the previously impossibly low personnel ceiling could be raised sufficiently to accommodate vital recruitments.

However, the continuing ceiling on Federal civil service salaries, in the face of sharply rising salaries elsewhere, has made such recruitments virtually impossible in most cases. The resort to the IPA mechanism may provide a short-term alternative, but it is hardly a solution to this most pressing problem. Notable are our failures, so far, to recruit a director for the Stroke and Central Nervous System Trauma Program or a chief for the intramural Surgical Neurology Branch. In the latter case, for example, the gap between \$60,000 plus salaries in academia and the \$37,800 ceiling at NIH has frustrated the efforts of our search committee and staff.

The final component in our reorganization was realized with the selection of Dr. George Murray, a "graduate" of the NIH executive development program, to be director of the NINCDS Office of Program Planning and Evaluation. When staffed and fully operational, this unit will provide to program directors, other top staff, and our outside advisors the analyses of past and current operations and the budgetary and programmatic impacts of future proposals that will be needed to allocate effectively and optimally the available resources.

After nearly 20 years as NINCDS executive office, Mr. Eckart Wipf retired at the end of 1975, to be succeeded by Mr. Paul Waugaman, a former Institute administrative officer and also a product of the NIH executive development program. The organization of our Intramural Program has been streamlined by subdividing it into a laboratory research division of 10 laboratories under Dr. Richard Irwin and a clinical research division of 7 branches under Dr. Donald Calne.

Three off-campus, intramural operations now exist. One is at the Marine Biological Laboratories at Woods Hole, Mass., where two of the three sections of the Laboratory of Biophysics now reside, under Dr. William Adelman, chief of the laboratory. This move recognized the special needs in this program that could be provided more efficiently by full-time work at the marine facility rather than annual treks back and forth. A special visit in March 1976 to these units by the Board of Scientific Counselors has assured us of the viability of this arrangement.

The second unit is at the Frederick Cancer Research Center at Fort Detrick, Maryland, where a significant portion of the primate holdings and slow-virus transmissibility studies of the Laboratory of Central Nervous System Studies are housed. The present temporary quarters will soon be vacated for the Institute's newly renovated building 376, due to be occupied by November 1976. The move should significantly enhance the resources and research potential of this program.

Finally there is the research facility on Guam, established in 1959 to study in the native Chamorro population the extraordinarily high incidence and prevalence of amyotrophic lateral sclerosis (ALS) and Parkinson-dementia, which together account for 10-20 percent of all deaths in this group on Guam. Active research on immunological factors and experimental therapies continues. But the big news this year is the survival of the unit and its patient population during typhoon

Pamela, which devastated the island for two days with winds in excess of 140 m.p.h. The NINCDS staff and consultants on the island deserve very special commendation for their spontaneous response to the emergency, both in safeguarding the research facility and records as well as ensuring the safety and well-being of nearly 100 patients in the program scattered over the island.

Conferences and Commissions

During fiscal year 1976, the NINCDS supported wholly or in part a number of conferences or workshops. In line with Congressional interests, a workshop on autism was held at NIH in February 1976 at which leading experts in research and clinical fields met to discuss current concepts and to recommend future direction for research. A report of the proceedings is in press. A similar workshop on ALS, supported by non-NIH sources but with major NINCDS participation, was held in Los Angeles in November, with a report also in press.

The 10th Princeton Stroke Conference took place in January 1976, with a particularly provocative program that included sessions on atherosclerosis, aging factors, and results of epidemiological surveys in Japan, Hawaii and California. The Japanese study was especially interesting because it documented relative prevalences of hemorrhagic and occlusive forms of stroke comparable to that in the American population. Moreover, hypertension proved to be the principal risk factor, whereas hypercholesterolemia (clearly a factor in myocardial infarctions in the same population) proved not to be a significant risk factor for stroke.

At an international conference in Montreal on cerebral edema, supported by non-NIH sources but with major NINCDS participation, recent clinical observations by German investigators were striking. They carried out long-term monitoring of intracranial pressure changes measured by implanted sensors, which emphasized the importance of barostabilization in brain tumor patients, and they demonstrated the efficacy of high doses (10-fold higher than usual) of dexamethasone in head injury patients. These and other reports indicated a significant assault on problems of cerebral edema and ischemia, but they also highlighted the fact that essentially all the clinical research data came from European investigators. As discussed at the conference, there is real concern over the constraints imposed on North American research by the human experimentation and malpractice problems.

This year marked the start of the Institute's participation in the World Health Organization program of collaborating centers for research and training in the neurosciences. The NINCDS is currently one of seven designated centers, the others being in Montreal, Strasbourg, Marseilles, Moscow, Mexico City and Ibadan (Nigeria). At a preliminary meeting in Geneva in January 1976, three NINCDS staff met with other center representatives and WHO staff to prepare an agenda for the third consultation on the program held in Montreal in April. In Geneva we received briefings on WHO from Dr. Mahler, director-general, and

Dr. Lambo, deputy director-general, and briefings on WHO programs in cardiovascular disease, malnutrition, and fellowships. Subsequently at the Montreal consultation the seven center directors developed protocols for collaborative studies on stroke and on epilepsy and for training programs. Concurrence was obtained with the plan for NINCDS to be responsible financially and programmatically for training annually in the U.S. six to eight fellows nominated by developing countries and selected by a collaborating-centers review panel. Progress on the other initiatives taken at the Montreal consultation may be expected during this year. The designation of additional collaborating centers in Africa, Asia and South America is planned by WHO, and the director, NINCDS, has agreed to serve as special consultant to travel to and review for WHO a number of such potential centers.

After almost two years of desultory negotiations with Soviet authorities, a three-man delegation from the USSR on spinal cord injuries arrived here in May 1976. This was partially in response to recommendations by the NANCDS Council subcommittee on central regeneration to evaluate Soviet reports of enzyme therapy in the acute stages of cord injury. Two conferences were held at which the Soviet visitors presented clinical and experimental data, and visits were arranged to appropriate U.S. laboratories and centers. The data from the Soviet clinics provided results that did not differ from those already available in the United States. Soviet emphasis on very early definitive treatment and aggressive rehabilitation did not produce outcomes significantly different from results of "unselected" series in the U.S. However, the experimental studies on spinal rats treated acutely with local instillations of hyaluronidase and/or trypsin enzyme preparations clearly seemed to minimize scarring at the site of cord transection and possibly improved the extent of motor recovery below the transection. The observations warrant independent repetition of the same experiments and extension to other species. One predictable result of the publicity attending the visit of the Soviet scientists has been a substantial upsurge in public pressure for more funds for more research on paraplegia and central regeneration.

Analogous pressures resulted in legislation last summer establishing commissions for epilepsy and for Huntington's disease. Although the Institute and NIH moved quickly to charter the two commissions and to nominate members, delays at higher levels prevented meetings until April 1976 for the epilepsy commission and July 1976 for the Huntington's disease commission (with one member still to be appointed). Thus, the programmatic and budgetary impacts of these commissions will not be fully felt until fiscal year 1978.

The general problem of commissions deserves comment. Two years after the multiple sclerosis (MS) commission submitted its report, the Institute has been challenged by the National Multiple Sclerosis Society directly in a series of meetings and indirectly through Congressional contacts in terms of the adequacy of its response to the recommendations. Despite the fact that the Institute had received none of the funding specifically recommended by the commission, the NINCDS appeared to be

placed in a position where it would be criticized whether it did nothing or tried to implement recommendations within available resources at the expense of other programs. Moreover, the issue surfaced of whether or not research is directly relevant to MS; despite a consensus that one could hardly be very specific in a disorder for which no cause, no specific diagnostic procedures, no preventive measures, and no treatment exist, and which clearly involves virological, immunological and genetic factors. These problems are compounded by the pressures created more recently by the diabetes commission. Obviously careful attention must be given to the impact on program balance and on limited budgets that special interest pressures, especially commissions, create. For this reason, the NANCDS Council agreed at its June 1976 meeting to create subcommittees to consider the recommendations of the MS and diabetes commissions and their implementation by the Institute. In addition to Council members, several ad hoc consultants have been carefully selected for each subcommittee. Their reports due in January 1977, will form the basis for the reports requested by the Congress at the time of the FY 1978 appropriations hearings. We anticipate utilizing a similar approach for recommendations of the epilepsy and Huntington's disease commissions.

Extramural Programs

An overview of the entire NINCDS extramural programs for fiscal year 1976 indicates that there were 1735 awards totalling \$110.8 million. Of these 1200 were research grants (79 program project or center grants and 1121 individual grants) at \$90.1 million (\$30.2 and \$59.9 million, respectively), plus 124 contracts at \$10.8 million, and 416 fellowship and training awards at \$9.9 million. These accomplishments should be tempered by the realization that there was an 8-month delay after the beginning of FY 1976 before the appropriation for grants and contracts became available, and legislative authority for training programs was not renewed until nearly the end of the regular fiscal year. In the past five years we have experienced a 45% increase in the number of grant applications, with a rise in approval rates to 65% of the total, reflecting mostly the increasing number of resubmissions. During fiscal year 1976 only about 25% of the competing approved research grant applications could be funded. The implications of these delays and constraints for problems of program management should be self-evident.

Some of the developments recorded for each of the five extramural program areas have been selected for brief mention. In the Stroke and Central Nervous System Trauma Program, support was provided for 184 grant and contract awards totalling \$17.8 million. This included 15 stroke centers, 6 spinal cord injury centers, and 5 head injury centers. Much of the research continues to focus on the central problems of ischemia and edema, and the roles of potassium leakage, transmitter imbalances and astrocytic swelling. New developments in monitoring regional blood flow, perfusion and metabolism appear promising, some of which may be exploited in conjunction with proposed comprehensive stroke programs designed to correlate management in the acute phases with rehabilitation outcome. In experimentally induced cerebral vasospasm,

studies by scanning electron microscopy have dramatically demonstrated the formation of mural thrombi. It remains to be determined whether these could relate to the symptomatology in transient ischemic attacks (TIA). A better understanding of the significance of the TIA for stroke is being sought in a planned, prospective, community study. Additionally, collaborative studies in India and proposed in Nigeria may contribute to this question as well as identifying other risk factors more definitively. The Nigerian study would complement a domestic study on stroke in black populations. A collaborative study of the efficacy of superficial temporal-to-middle cerebral artery anastomosis in TIA is in the planning stage. In cord and head injury patients a controlled evaluation of steroid therapy is also being planned. A number of projects relating to central regeneration were funded this year, in accord with the NANCDS Council subcommittee recommendations. Regardless of who deserves the credit, it is gratifying to note that the latest statistics on mortality in the U.S. record significant decreases for both stroke and trauma. We suspect that much of the improvement reflects better management during the acute episode, as a consequence of research supported by NINCDS, other NIH institutes and the VA.

In the Communicative Disorders Program there were 311 grant and contract awards, including 16 program project and center grants, for a total of \$19.55 million. Following the phase out of the information network center at Johns Hopkins last year, there has been a careful evaluation of National Library of Medicine capabilities. With development of an appropriate Thesaurus it seems likely that an effective substitute resource can be developed. Institute staff and consultants contributed importantly to the deliberations of the federal hearing aid task force, and an NINCDS-sponsored workshop on visual and tactile aids for the deaf summarized the current status and future needs in this field. Increasing attention is being focussed on speech and language areas, as the whole problem of learning disabilities continues to be of concern. Progress on a broad front continues in the auditory (cochlear) prosthesis field--evaluation of previously implanted patients has been completed and the report is awaited, while development continues of more versatile stimulators and electrode arrays for experimental evaluation.

These latter studies are shared with the neural prosthesis projects in the Fundamental Neurosciences Program. During FY 1976 this program supported altogether 367 grants and contracts, totalling \$20.7 million. It is our most diversified program aimed at the acquisition of fundamental new knowledge along a broad front, with little obvious direct relevance to specific disorders. All program areas support fundamental research but in this specific program the orientation is more clearly to basic principles with broad applicability. Only a few of the many advances can be mentioned. Research on receptors has been most fruitful, especially the isolation and purification of cholinergic (acetylcholine) receptors to permit a variety of important experimental and clinical studies. Equally intriguing is the identification of

specific opiate receptors on neurons in certain brain areas together with the discovery of simple, endogenous pentapeptides with opiate-like properties elaborated by the receptor neurons. Correlations have been made between these findings and the phenomena of drug tolerance, habituation and withdrawal. The observations provide us with hitherto quite unexpected properties of certain central neurons and presage not only breakthroughs for the drug-addiction problem but also new approaches to the whole problem of pain mechanisms. Other major areas concern the plasticity of the central nervous system--i.e., the ability to delete or modify old connections, develop new connections and reshape neural networks as part of the process of development, adaptation, learning and perhaps regeneration--and the importance of local circuits served by short-axon or axonless neurons interacting through dendritic networks to modulate central activity. These neurons have been thought of as comprising a "second" nervous system which is likely to have profound implications for our understanding of central processing and integrative functions.

By far the largest of the NINCDS extramural programs is the Neurological Disorders Program, comprising in FY 1976 a total of 873 grant and contract awards, including 27 program project and center grants, amounting altogether to \$52.7 million. In the field of epilepsy, two additional comprehensive epilepsy programs were approved and funded--one at the University of Washington, Seattle, covering the states of Washington, Alaska, Idaho and part of Montana, and the other at the Medical College of Georgia, Augusta, covering the state of Georgia. These bring the total to five, with the other three having exhibited good progress during their first year. Another new anticonvulsant drug, clonazepam (Clonopin) was approved for marketing, with special indications for absences (petit mal) seizures, and another promising new drug, dipropylacetate (Valproate; Depakine), used extensively in Europe for the past 10 years for generalized seizures, is now under evaluation in the U.S. by NINCDS grantees and contractors. In addition, the Institute is engaged with industry in an extensive drug screening program in a search for more new anticonvulsants, and has contracted with the National Bureau of Standards to develop drug reference standards for use in quality control of clinical blood level analyses. In data drawn from the collaborative perinatal project, analyses of cases of febrile seizures in early childhood indicate that seizures persist only in those patients with demonstrable neurological deficits at the time of the original febrile seizures. Evaluations of chronic cerebellar stimulation as a means of seizure control have suggested benefit to some patients, but more experimental studies are needed in view of findings in several clinics of significant losses or near absence of Purkinje cells in cerebellar biopsy samples taken at the time of implanting the cerebellar electrodes. The implications of this finding for the stimulation procedure as well as for epilepsy generally deserve careful evaluation.

In a number of other disease areas there has been encouraging progress also. For Parkinson's disease, the dopamine agonists, such as bromocriptine, are proving more effective than L-DOPA, with less unpleasant side-effects. For Friedreich's ataxia, evidence of an

enzymatic defect at the key, pyruvate oxidase stage of glucose metabolism, and benefits from a ketogenic-type diet, may signal progress in understanding this puzzling disorder. For muscular dystrophy a number of investigators are assessing several apparent membrane defects as a possible basis for the disease. Myasthenia gravis now seems clearly to be an autoimmune disease caused by circulating immunoglobulin antibodies to the acetylcholine receptor at the neuromuscular junction. It is interesting that these studies have demonstrated that there are pre-synaptic as well as post-synaptic acetylcholine receptors at the junction, both of which are blocked by the circulating antibodies. The pre-synaptic receptors may well provide for modulation of nerve activity. In Huntington's disease, the finding of several biochemical abnormalities may presage a beginning to our understanding of the molecular basis for this disease and hopefully may lead to antenatal screening procedures. Clues for Alzheimer's disease and the senile dementias are also beginning to emerge. The characteristic neurofibrillary tangles have been found to comprise the usual tubulin protein but are misassembled or overproduced, perhaps on the basis of gene derepression and/or a slow virus effect. The latter possibility is also suggested by the fact that the typical neuritic or senile plaques, which contain a core of amyloid, appear identical with similar plaques specific in the brains of scrapie-infected animals. Establishment of a link between slow virus infections of the central nervous system and the senile dementias would be a major breakthrough for a problem of serious dimensions for a major proportion of our older citizens.

The neurovirology field in general continues to be very active, with increasing attention given to defective and latent viruses. Characterization of the measles virus responsible for subacute sclerosing panencephalitis (SSPE) and of its RNA is well along. One of the latent viruses, herpes, has been shown to enter autonomic as well as sensory ganglia via retrograde axoplasmic transport from genital infections. The J-C virus, one of the papova group responsible for progressive multifocal leukodystrophy (PML), is clearly also tumorigenic for the central nervous system. New techniques developed for demonstration of latent neurotropic viruses include cell fusion and co-cultivation of cells in tissue culture, use of DNA inhibitors to induce viral synthesis, and resort to neonatal animals where susceptibility is enhanced. Some of these studies are directed at ALS, as a potential slow or latent virus disorder. Studies continue on characterization of the C-type virus particle found in the Guamanian brain material (both ALS and controls), and some evidence for immunological factors in ALS has emerged.

The closely related problem of multiple sclerosis (MS) represents a very active research area. In the experimental allergic encephalitis (EAE) animal "model", pretreatment of animals with myelin basic protein aborts the development of EAE, and EAE animals exhibit increased activity of the enzymes which degrade myelin basic protein. A number of immunological tests have been reported, including specific oligoclonal antibodies in CSF, lymphocyte migration tests and the like,

but all have suffered from imprecision and difficulty of replication. An earlier report of the isolation of a transmissible factor from MS brains was confirmed this year, but subsequent studies have been somewhat equivocal. Evaluation of possible genetic factors in MS has continued with attention to the histocompatibility antigen profiles in the sera of matched controls and of MS patients in family "clusters" and in the MS focus on the Orkney and Shetland Islands, where incidence and prevalence rates are the highest in the world.

The NINCDS Office of Biometry and Epidemiology (OBE) is responsible for support of the epidemiological study on MS in the two Scottish islands. The study there has almost finished, with a major aspect being the analyses of the histocompatibility antigen profiles. The OBE staff has initiated under contract several other surveys within the United States to study the incidence, prevalence and socioeconomic cost factors for multiple sclerosis, brain tumors, spinal cord and head injuries, and stroke. These studies are at the feasibility or pilot phases and are closely monitored by consultants and advisory committees. A fifth survey for epilepsy is in the planning stage. The use of sophisticated population sampling techniques is being pioneered in these disease-oriented surveys. Other NIH institutes and the National Center for Health Statistics are watching the NINCDS experiences closely, and our OBE staff is collaborating with these other units to capitalize on these techniques for longer-range statistical purposes. These and other studies have been strengthened by the recruitment of Dr. Bruce Schoenberg, a well trained neurologist and neuroepidemiologist, to head the epidemiology section in OBE.

Intramural Program

Research progress in the various intramural laboratories and branches of the Institute continues to be exceptionally fruitful. Of the total of \$30.9 million allocated by the Institute to direct operations, \$19.5 million (or about two-thirds) funded intramural research on 227 research projects by a staff of nearly 400 permanent and temporary or visiting personnel. The three newest components, the Laboratory of Neuro-otolaryngology, the Neuro-immunology Branch, and the Laboratory of Neuropharmacology are now fully operational, and the EEG Branch has been reorganized into a Clinical Neurosciences Branch, incorporating the previous EEG and neurophysiology units plus a section on functional neurosurgery. This latter arrangement brings together all the intramural clinical epilepsy research in one unit.

In contrast to the extramural projects which can only be selectively summarized in the appropriate sections of this annual report, a summary of each of the 227 intramural project is appended for perusal. Accordingly only a few highlights are considered here. As an indication of the extent of clinical research activities, 1310 EEG recordings were carried out by the Clinical Neurosciences Branch, with two-thirds on NINCDS patients and the remainder mostly on patients from NIMH and NCI. A number of these were recorded on the wards or in intensive care units.

During FY 1976 the Medical Neurology Branch, for example, admitted 317 patients for 7962 patient days, recorded 1135 outpatient visits, examined 450 muscle biopsies, and provided 490 neurological consultations to other NIH Clinical Center units. Also during this year the computerized axial tomography instruments (CT head and body scanners) were installed and became operational. The NINCDS defrayed part of the cost of the first head scanner, and purchased a second head scanner for research use by the staff of the Institute's section on neuroradiology. As expected these instruments are already overtaxed by the number of demands for scans from the NIH and from other federal facilities.

For a variety of myopathies, prednisone continues to prove therapeutically effective. In myasthenia gravis, its efficacy appears to correlate with a relatively selective depression of circulating T-lymphocytes. The combination of azathiaprine, another immunosuppressant drug, and prednisone has markedly alleviated the calcification of muscle and skin in some severe cases suffering from the polymyositis/dermatomyositis disease complex. The use of tissue culture of muscle cells has provided considerable insight into several myopathies. By utilizing this approach, acid maltase deficiency is the first to be established as a primary myopathy, since the biochemical and morphological characteristics can be "reincarnated" in culture. Similar observations have now been made for phosphofructokinase deficiency myopathy. Also the previously observed recovery of enzyme activity in cultures from glycogen phosphorylase deficiency myopathy has been reconfirmed.

New methodologies have greatly facilitated research in a variety of projects. A micromethod has been developed for determining the fluid content of small tissue samples by obtaining the specific gravity in a kerosene-bromobenzene liquid column. Thus, the extent of edema can be evaluated in very localized brain or spinal cord areas. Using this method, it has been shown for the first time that there is significant local tissue edema within less than 10 minutes after the onset of cerebral ischemia. With the use of horseradish peroxidase (HRP) which is taken up by nerve endings and transported by retrograde axoplasmic flow to neuronal cell bodies where it can be visualized electronmicroscopically, it has now been shown that HRP injected intravenously is taken up from muscle capillaries to enter the central nervous system. This circumvention of the blood-brain barrier carries significant implications for the ease of systemic entry of toxins (tetanus) and viruses (rabies, polio) which are known to be carried into nerve cells by the retrograde axoplasmic route. A similar HRP technique has also proved useful in mapping the distribution of spinal motor neurons and interneurons serving specific muscle groups. Such information may be applicable to the ALS problem. By using freeze-fracture electron-microscopic techniques specialized tight junctions have been demonstrated between the apices of hair cells and supporting cells separating perilymph from endolymph in the cochlea. These junctions are more extensive and tighter than any seen in other tissue sites so far. Presumably they may play a role in mechanoreception and in mechanisms responsible for the special ionic composition of the cochlear fluids. Companion studies on key enzymes have demonstrated

carbonic anhydrase to be a major cochlear enzyme, comprising about 1% of the membrane protein lateral to the endolymphatic space. Other enzyme data indicate which transmitter agents are or are not likely to mediate the activity of various cochlear and auditory nerve fibers.

Freeze-fracture electronmicroscopic techniques have also been crucial in elucidating details of synaptic transmission. Institute scientists have been able to show that at the nerve ending botulinum toxin produces its paralytic effect by blocking exocytosis of the synaptic-vesicle content of transmitter, probably by preventing the entry into nerve terminals of calcium ions necessary for initiating vesicle exocytosis. In contrast the venom of the brown widow spider exerts its toxicity by promoting total exocytosis of all vesicles simultaneously, probably by facilitating the entry of calcium ions. Experimentally the venom can overcome the effects of the toxin. The budding of viruses from cells is a closely analogous process, and the same freeze-fracture electronmicroscopic techniques have been used to study cells chronically infected with the SSPE measles virus. The investigators have been able to pinpoint the specific defect in assembly of the viral membrane preliminary to maturation and budding, thus accounting presumably for the slow virus infection in SSPE. Other studies showed that sera from SSPE patients when mixed with complement caused destruction of brain cells and that sera or CSF from SSPE patients inhibited the development of cellular immunity to the measles virus, presumably because of the presence in these fluids of a "blocking" factor.

Virological research is active along a broad front. Intramural scientists have documented the transmissibility of the Creutzfeldt-Jakob agent via transplanted tissues (e.g., cornea) and have established that the agent survives in tissue samples preserved in formalin for as long as 8 months. These hazards to transplant recipients, surgeons, pathologists, and the like are being widely publicized. Another high incidence focus of ALS has been added to those on Guam and the Kii peninsula in Japan. The latest is in western New Guinea and reinforces the conviction that a slow virus type of agent could well be involved. Institute scientists have made an especially valuable contribution by identifying Patas monkeys as the asymptomatic carriers of simian hemorrhagic fever virus, which frequently decimates colonies of rhesus (macaque) monkeys. Thus preventive measures are now possible by isolating Patas stock and protecting rhesus colonies with an immunostimulant preparation. In mice with latent herpes infections in dorsal root ganglia, injury to the peripheral nerve reactivates the virus. This "model" may prove useful in delineating factors responsible for latent virus reactivation in a variety of disorders.

Among the most provocative of the viral studies are those on defective interfering viral particles (DIP), particularly those formed by RNA viruses of the myxo-, paramyxo- and rhabdo- virus families. NINCDS intramural scientists have found that the DIP's comprise different parts of the normal viral genome but all contain the same 3' and 5' end of the RNA sequence. With one or both of these ends the

DIP's bind to the viral replicase to reduce the amount of normal RNA that can be made. Moreover, since the messenger RNA needed for viral protein synthesis is transcribed only from normal viral RNA, the total number of viruses (infective and non-infective) into which RNA molecules can mature is reduced as a result of this interference with viral protein synthesis. The persistence of virus in infected cells is not related to virus-derived DNA but to the continued presence of viral RNA itself, kept from expressing itself by autoinference from the DIP's. To detect the presence of such RNA molecules, the investigators developed a new technique by which DNA complementarity to viral RNA could be produced with reverse transcriptase, so that DNA hybridization techniques could be used to ascertain whether certain viral nucleic acids are actually present. Since these RNA viruses include measles, influenza, rabies and others of clinical neurological importance, these studies have widespread implications.

Clinical results with enzyme replacement therapy for one of the lipid storage diseases, Gaucher's disease, continue to be surprisingly promising. An efficiency of 40% has been estimated on the basis of the ratio of glucocerebroside cleared from the liver (ascertained by biopsy) to the unit activity of the enzyme administered. Experimentally the injected enzyme persists in liver cells five-fold longer in animals anesthetized or sedated with barbiturate. Regardless of the ultimate applicability of these studies, some remarkably provocative observations are being recorded. In another of the lipid storage diseases, Krabbe's globoid leukodystrophy, diagnostic and screening procedures will be considerably simplified as a result of the synthesis by NINCDS scientists of a chromogenic analog of the galactocerebroside substrate for the affected enzyme.

In a follow-up of previous findings that there is a characteristic glycoprotein in myelin, NINCDS investigators have now localized it as a surface component of myelin, where potentially it could serve as a receptor or site of attachment for various toxic agents including viruses. The whole field of cell receptors seems about to undergo a major advance. Evidence is accumulating that dopaminergic receptors may prove vital to a number of hormonal functions. Thus, in the use of dopamine agonists, patients with Parkinson's disease exhibit a low growth hormone response, suggesting an attenuation of the sensitivity of their dopamine receptors. In Huntington's disease the dopamine agonists, such as bromocriptine, exacerbate the clinical symptomatology, an observation indicative of probable hyperactivity of dopaminergic systems. Interestingly in lead poisoning of the central nervous system the increased release of dopamine and inhibition of its re-uptake suggests that the toxic effect of lead may involve disruption of the dopaminergic system.

Surely, some of the most intriguing observations in the receptor context are those concerned with the role of specific gangliosides as cell surface receptors for trophic hormones in a variety of tissues. Intramural scientists have demonstrated, for example, that thyrotropin, the thyroid stimulating hormone, reacts with the Gp1b ganglioside, whose structure as such or as part of a glycoprotein is naturally

present in thyroid cell membranes, where it presumably represents the natural receptor for the hormone. The hormone is a glycoprotein of known structure, and other hormones such as luteinizing hormone and human chorionic gonadotropin have structural homologies with thyrotropin. Thus, the effects of these other glycopeptides may also be mediated by gangliosides in the cell membranes of target cells. Of special interest is the homology with cholera toxin. By using in tissue culture a strain of cells lacking gangliosides, NINCDS investigators showed that the addition of as few as 17,000 molecules of G_{M1} ganglioside per cell conferred sensitivity to the toxin, whereas normally such cells remained completely unresponsive. In addition, the studies demonstrated that the binding of one subunit of the toxin molecule to the membrane ganglioside was a prerequisite for the other subunit of the toxin molecule to be able to penetrate into the cell and exert its toxic effects. It is remarkable how far this work has come from the relatively "simple" beginnings of the study of ganglioside accumulation in Tay-Sachs disease to observations on such diverse involvement of gangliosides as in the enzymatic alterations of cultured cells transformed by oncogenic viruses, in the effects of lipophilic acids in cellular morphology and membrane transport, and in receptor functions for hormones and toxin molecules.

Conclusion

In these and many other examples cited above, projects in the NINCDS intramural program are characterized by highly effective collaboration among several laboratories or branches within NINCDS and/or other units at NIH or elsewhere. As research problems and the techniques to study them become more complex, this phenomenon of collaboration is increasingly common and is certainly a major factor in the productivity of the program. Space does not permit individual acknowledgement here of the many non-NINCDS scientists who have contributed in this way, but a special note of appreciation is in order.

The growing complexity of research also places greater demands on us to disseminate and explain the results and applications. The Congress has now taken special cognizance of the need to address this problem. Within the Institute the major responsibility falls on the Office of Scientific and Health Reports (OSHR), and details of their efforts are provided in their report appended below. Among many examples during FY 1976 are the 3000 inquiries from individuals about specific neurological or communicative disorders, response to over 400,000 requests for NINCDS publications, and provision of video tapes for viewing by more than 85,000 physicians. The time has come to give this program the same managerial recognition as other NINCDS programs. Accordingly we plan in the coming year to expand the responsibilities of our Scientific Information Program Advisory Committee (SIPAC) to maintain a continuing overview of OSHR activities and to advise us on how to do the job even more effectively.

No research program is better than the personnel responsible for its management and implementation. Despite the severe constraints on staffing under which the NINCDS has labored over the past several years, we are blessed with an extraordinarily dedicated and able staff. A

major ingredient has been an effective equal employment opportunity (EEO) program. The NINCDS has been in the forefront at NIH in the implementation of EEO principles and programs. There is still much to be done, but there is also much credit deserved by the EEO personnel and other Institute staff. Unless we can sustain these efforts in the immediate future by enlightened personnel ceiling, recruitment, promotion and pay policies, the Institute's abilities to carry out its mission could be seriously impaired.

Such considerations have a special relevance this year, in light of the recently released report of the President's Biomedical Research Panel. At the end of the report the panel lists five areas for future emphasis: population research, diabetes, genetic diseases, environmental and toxicological research, and neurobiology. Of the first four, all but population research now directly involve major neurological problems. For neurobiology, the panel's conclusions deserve reiteration here:

"Perhaps the ultimate challenge to biomedical research, representing the very pinnacle of our understanding of the human organism, lies in neurobiology: how the brain and the nervous system develop, how they function in health and disease, how thought occurs, how memory is stored, how we reason, how we are motivated, and how we interact with our physical and social environment.

We hope that man will one day have a better understanding of himself and live more harmoniously with his fellow man. Thus, the study of brain and mind deserves greatly increased attention not only in the programs of the NIH and the ADAMHA but also from the many different disciplines of biomedical and behavioral science, as well as such fields as mathematics, linguistics, and the communicative sciences. THIS PANEL COMMENDS NEUROBIOLOGY AS A COMPELLING LONG-RANGE INTEREST WORTHY OF NATIONAL ATTENTION."

ANNUAL REPORT
For Period July 1, 1975 through June 30, 1976
Office of Program Planning and Evaluation
Office of the Director
National Institute of Neurological and
Communicative Disorders and Stroke

The Office of Program Planning and Evaluation, as staff advisors to the Director and the Institute on program development and analysis, assists the Director and the Institute program managers in the analysis and evaluation of their programs, and in the development of strategic and operational program plans to meet the long-range goals and immediate objectives of the Institute. The Office provides staff support to facilitate, coordinate, and integrate program planning, analysis, and evaluation efforts in the categorical program areas and provides the Director and the Executive staff with assistance in setting, articulating and actualizing the goals of the Institute programs and strategies for meeting those goals. The Office has developed with the staffs of program areas of the Institute, a process to prepare annual implementation plans which will form a basis for resource allocation decisions and a development of budget requests for future years.

The Office has also accepted the responsibility to develop, for the Institute, an integrated Program Information System. This will combine scientific program summary data with fiscal and management data into an integrated Institute-wide data base which will provide necessary information required to effectively and efficiently plan and manage program activities at all levels of the Institute. In order to assist the Institute in developing a methodology for, and system design to support, the efficient input, storage, and retrieval of the scientific data including the content and anticipated product of the Institute research program efforts, evaluation funds have been provided from the Assistant Secretary for Health to support this project. In collaboration with, and with the assistance of, the Office of Biometry, a review-and-analysis of Institute program and management information needs is being carried out. Further, a review-and-analysis of available information data systems both within and outside of the Institute is also underway. An RFP was issued in late spring to secure the technical support to develop a system design and programming specifications for the proposed Program Information System. Award of a contract is expected during the transition quarter.

The Office has been a focus of assistance to the Office of the Director, NINCDS, and to all parts of the Institute, in (a) gathering and coordinating input for, and in developing special reports (oral or written) and issue papers concerning the Institute's program efforts in specific areas including population research; support of clinical application, control/demonstration, basic research; technology assessment; studies on violence; genetics; international studies; studies on children; studies on arboviruses;

Indian health survey; clinical trials; studies on multiple sclerosis; research on aging (statement on dementia); and studies on vision; (b) in reporting on interagency concerns such as NINCDS studies in heart/lung, cancer, diabetes, nutrition, and arthritis; (c) in working closely with all Institute elements in developing (with special assistance from Budget office) a number of major planning and budget documents including the Forward Plan (for both Fiscal Year '77 and Fiscal Year '78) projecting the Institute's objectives and resource needs through Fiscal Year '82, the preliminary Fiscal Year '77 budget for OMB, and the Evaluation Plan for Fiscal Year '76 in cooperation with OBE.

The Office provides a central management focus for the Institute's information service programs, which provide summarized literature review, bibliographic and reference to the scientific research and clinical community in fields related to the Institute's mission. A plan is being developed for the evaluation of these programs including the analysis of the options, and possible alternative methods of providing for the objectives of these services. It is hoped that this evaluation can, by mid-FY'77, provide to the Director, necessary information for the decisions concerning the supplementation and/or restructuring of these information services. One area of specific concern is information services in hearing, speech, and disorders of human communication. During FY'76, the contract with Johns Hopkins was discontinued and alternatives are being sought, together with the Communicative Disorders Program (see contract narrative NIH-NINCDS-71-2281).

In addition, the Office provides a central focus for the administration of foreign currency awards or P.L. 480 program. A special report on this program is attached.

The Office continued to work on the following research projects:

1. Study of Mental Retardation -- A record anchored retrospective study of mental retardation cases as diagnosed in Georgetown University Clinic with intent to discover etiological associations with selected prenatal variables (genetic, socio-economic, obstetric) as these are compared in index cases with sib and non-sib controls from the Georgetown Center as well as from the COLR Study. (See Annual Report Project Number ZOI NS 01144-14 OPPE.)
2. Public Health Implication Study -- A comparison of death rates (1959-1965, fetal, neonatal, infant mortality): (a) In the COLR populations, intra- and inter-institutionally, annually; (b) In the population of cities where these institutions are located and from which the COLR studies have been selected; and (c) In these two population samples. (See Annual Report Project Number ZOI NS 01146-14 OPPE.)
3. Study of Labor -- The pediatric outcome of vaginal deliveries in normal pregnancy compared along selected parameters with the outcome of elective CEsarian deliveries, with intent to discover, if possible, the effect of uterine contraction, on time and prematurely, on normal growth and development of the offspring. This study is reactivated during FY'77 in collaboration with NICHD; analysis and manuscript to be completed.

4. A Study of Comparative Health -- In selected ethnic U.S. groups, a survey of incidence and prevalence of MS, ALS, Parkinson's disease, etc., and other bio-psycho-social parameters to compare with adequate controls from samples of population of the same ethnic origin in other lands. The comparison will be made in a selected period of time and the analysis will hopefully reveal some insight into the mechanism (genetic or environmental) of production of these selected parameters. This study is in design.

5. Comparative Study of Schools of Thought in Medical Science and Practice -- (A study in medical care). In the U.S., allopathic medicine is thought and practiced side by side with homeopathic medicine, etc. Many systems of medicine are prevalent in India. In Lebanon, the American University has a faculty of medicine and the French University has another faculty, thereby offering a chance to compare French and American medicine. This has implications not only to the systems of delivery of medical care but also to the growing concern in training physician aides even in the developed countries. This study is in design.

6. Administration Research in Collaboration with NICHD -- This is a test of the hypothesis that authority and responsibility in any bureaucratic hierarchy are equal only in middle levels and that authority disproportionately increases the higher the level, and conversely, responsibility disproportionately increases the lower the level. This study is in design.

The Office also surveys, searches, excerpts and abstract-reviews medical literature as program needs arise; bibliographies and references are verified and select documents are put together utilizing MEDLINE, MEDLARS, SIDLINE and other computer systems.

The Office staff has also been active in a number of associated activities such as coordinating the Combined Federal Campaign, U.S. Bond Drive, the Committee on Science and Human Values, and NINCDS EEO Advisory Committee.

Finally, following the appointment in April of Dr. George C. Murray, as the Director of the Office, (NINCDS Deputy Director, Dr. E. Eagles, had been its Acting Chief) two additional professional staff members have been actively recruited, and should be appointed early in the transition quarter. These additional staff will provide for the expanding functional role and increased activities of the Office as they evolve, particularly in the areas of program analysis and evaluation, and in the development of the Program Information System. (Total staff 8; 4 professionals).

CONTRACT NARRATIVE
Office of Program Planning and Evaluation, NINCDS
Fiscal Year 1976

INFORMATION CENTER ON HEARING, SPEECH, AND DISORDERS OF HUMAN
COMMUNICATION (N01-NINCDS-71-2281), Johns Hopkins Univ., Baltimore, Md.

Title: Operation of an Information Center on Human Communications

Contractor's Project Directors: Michael Weiss, Ph.D., and Lois Lunin

There will be no continued funding for this project.

The Information Center was phased out of existence during the first six months of FY'76. All publication activities were halted, and activity was limited to closing down the Center.

Mailing lists and pertinent records were sent to NINCDS. Subscribers were given refunds, and requests for existing publications were forwarded to the Institute. The project officer took over the task of filling an initial set of requests for bibliographies. Data base magnetic tapes were transferred to the Institute.

The microfilm copies of original articles referenced in the Center's alerting bulletin were transferred to the NIH library, where they are now available to users of the library.

A cooperative effort has begun with the National Library of Medicine to improve both the indexing vocabulary and journal coverage by NLM in the communicative disorders field. Eventually it may be possible to utilize the NLM's capability to enhance retrieval of information on research results in communicative disorders. This effort will continue through FY'77.

Special Report
Foreign Currency Credit Award Programs
(PL 480)
Fiscal Year 1976

The foreign currency credit award or PL 480 Program is a program of research supported by United States owned foreign currencies which are not convertible to dollars. It is authorized by the Agricultural Trade Development Systems Act, 1954, Public Law 480, subsection 104K, which permits the use of foreign currencies, "to conduct and support scientific activities overseas, including programs and projects of scientific co-operation between the United States and other countries such as coordinated research against diseases common to all mankind, that are unique to individual regions of the globe, and promote and support programs of medical scientific research."

In Fiscal Year 1976, NINCDS had 22 ongoing projects. There was one project in Egypt, six in India, five in Poland, and ten in Yugoslavia. Funds are available in Pakistan and Tunisia, but we have no projects in these countries. The scientific content of the PL 480 research is complementary to other NINCDS research and covers both clinical and laboratory studies.

Each of the PL 480 projects must have a U.S. scientist to serve as a collaborating "Project Officer" who works with the foreign scientist in varying degrees. The role of the U.S. project officer varies from that of an active collaborator participating in the research to that of an informed sponsor. However, the project officer must each year review the expenditures and the progress report and advise the Institute whether the progress has been satisfactory. Of the 22 projects, six have project officers who are in the NINCDS. The other 16 project officers are in universities throughout the country.

Available funds for the Yugoslavian activities have been used up. New projects must be done on the basis of U.S. dollars matched by Yugoslav dinars. No great interest in doing this has been shown.

There is a large amount of money in Egypt available for PL 480 research projects, but there is difficulty in finding qualified people. The number one priority health problem is schistosomiasis, followed by infectious diseases (including meningitis). Egyptian researchers are interested in the neurological diseases, particularly accidents and injuries to the CNS. However, the facilities are very poor and modern equipment in the laboratories is scarce. Clinicians and scientists who have trained outside of the country are well trained and capable. They are limited by the lack of facilities and financial support. Also, there is very little infrastructure of technicians or facilities for repair and maintenance of equipment. This is a reflection of the fact that there is only about 40 percent literacy in the country.

In Poland, the funds available for use have been sharply curtailed and will be fully obligated by December 31, 1976.

The PL 480 program in Israel is totally phased out because all funds have been used. The current projects are as follows:

Egypt	03-006	Venoms of Poisonous Snakes
India	01-015	Neural Mechanisms in Regulation of Feeding Behavior
	01-016	Neurologic Investigations of Cerebrovascular Disorders
	01-024	Collaborative Neurologic Studies
	01-034	Neurotoxins from Plant Materials
	01-040	Polypeptide Constituents of Snake Venoms
	01-041	Effect of Protein Malnutrition on Brain Metabolism
Poland	05-002	Characteristics of Muscle Disease
	05-004	Biology and Pathology of the Neuron and the Glia
	05-013	Biology and Histochemistry of Gliomas
	05-027	Biochemistry of Myelination and Demyelination
Now	05-092	
	05-035	Brain Fine Structure in Rabbits with Hereditary Tremor
Yugoslavia	02-003	Neural Correlates of Behavior
	02-006	Histogenesis of the Forebrain in the Human Embryo
	02-008	Function of Cholinesterases
	02-015	Metabolism. Release and Uptake of 5-Hydroxytryptamine
	02-020	Function of Biosynthesis of Ribosomes
	02-023	Vestibular Influences on Cerebral Activity
	02-025	Neuropathology of Close Brain Injury
	02-041	Synaptic Transmission in Brain
	02-042	Brain Survival in Anoxia and Hypothermia
	02-090	Gene Transcription and Gene Expression in Sea Urchin Embryos

Project 01-015 is concerned with the central and autonomic control system modulating alimentary receptor activities and regulation of feeding behavior. Project 02-003 is a large program to understand the function of the nervous system by studying physiological and biochemical correlates of behavior. In conjunction with this study is the development of a major research source, the International Brain Research Laboratory in Yugoslavia.

Several projects are basically neurochemical or metabolism chemistry studies, but they are generally unrelated. Project 02-042 is a study of

the tolerance limiting role of metabolic events occurring in the brain at conditions of anoxia induced at different levels of hypothermia down to zero degrees Centigrade. Project 05-027 (now 05-092) is a study of the role of neuroglia cells in the myelination and demyelination with emphasis on the enzyme chemistry of the processes. This relates to human demyelinating diseases such as multiple sclerosis. Project 05-004 is somewhat similar and is a study of the glia of the nervous system and their function in diseases of the brain. Particular attention will be given here to the intracellular oxidation reduction enzymes. This project terminated in FY76, but a new proposal is anticipated. The control and regulation of protein synthesis in biological systems is the focus of Project 02-020.

There is one neuropathological study, Project 05-035, which is a study of the fine structure of the brains of rabbits with hereditary tremor. This tremor is similar to that seen in Parkinson's disease and this study may have some bearing on that disease.

There are a number of studies which relate to the neurological diseases of children. One study, Project 01-024, is both biochemical and clinical and deals with the enzymatic processes involved in the sulfation and desulfation of glycosaminoglycans and cerebroside sulfate. These biochemical mechanisms have been studied in specific metabolic disorders of children. Using material from these patients the scientists working on this project have identified specific enzyme defects. There is a study that is related to nutrition of the brain. Project 01-041 studies the effect of protein malnutrition on the brain, metabolism and development.

There is a project relating to trauma of the nervous system. Project 02-025 is a neuropathological study of closed brain injuries to determine the topographic distribution and histopathology of closed head injuries.

Project 01-016 is a study of clinical angiographic and anatomic aspects of cerebrovascular disease in India. Project 05-002 is an extensive multiple disciplinary program for the study of muscle disease in Poland. Project 05-013 is a study of the biology and histochemistry of gliomas using tissue culture and immunochemical methods. Project 02-090 is a new study on gene transcription and gene expression in Sea Urchin Embryos.

There are projects concerned with naturally occurring neurotoxins. Of these, Project 03-006 is concerned with obtaining material of the poisonous animals such as snakes from the Middle East, North Africa, and as far South as the Union of South Africa. Biochemical and physiological studies will be carried out on the purified toxins which will also be developed. Project 01-040 is a similar study being carried out in India, studying the poisonous snakes of India. Project 01-034 is on neurotoxins from plant materials, and is focusing on toxicity of B-disminopropionic acid (ODPA) which is extracted from the seeds of Lathyrus Sativus in India. This will be studied in connection with the development of human lathyrism.

There are three projects concerned with synaptic transmitters. Project 02-008 is to determine the role of the acetylcholinesterases in the acetylcholine system transmission conduction of excitation. There are two others which are somewhat related to the study of the metabolism release of 5-hydroxytryptamine and its function as a transmitter in the brain. Projects 02-015 and 02-041. These three are in Yugoslavia.

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

An Instrument for the Conduct of a Retrospective Study of Seizures,
Cerebral Palsy, Mental Retardation (M.R.) and Other Neurological and
Sensory Disorders of Infancy and Childhood.NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Z. A. Shakhshiri, M.D. Special Assistant OPPE, NINCDS
OTHER: Mr. Ernest Harley Systems Analyst EBRP, NICHHD

COOPERATING UNITS (if any)

LAB/BRANCH
OD-NINCDS

SECTION

Office of Program Planning and Evaluation

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

.25

PROFESSIONAL:

.20

OTHER:

.05

SUMMARY OF WORK (200 words or less - underline keywords)

A retrospective instrument has been designed (index cases, with sib and non-sib controls) for record-anchored Study of Mental Retardation (cases selected from Georgetown (G.T.) University Hospital Specialty Clinic) as a pregnancy outcome. Ten hypotheses were being tested, related to the biological and psycho-social mechanisms underlying the occurrence of damage in the growing white child by age 5 years: anoxia, toxic influences on brain, metabolic influences, trauma to the head, infection of brain, dehydration of child, genetic or familial factors, socio-economic status, prematurity, and nutrition. Heart disease, prenatal hypertension, breech presentation, bleeding, Cesarean Section (C.S.) and Cephalopelvic Disproportion (C.P.D.), were noted more frequently in mothers of M.R.; and meconium, B.W. <2500, and neonatal head injury more frequently in M.R. children themselves, than in the corresponding sib and non-sib controls. Findings from G.T. data base are being compared to findings from prospective Collaborative Perinatal Research (COLR) data base, using the same retrospective instrument. In view of less incomplete data in the latter, this comparison would confirm or invalidate the initial findings from the former.

Project Description:

Objectives: Design an instrument for the conduct of a retrospective study of seizures, cerebral palsy, mental retardation and other neurological and sensory disorders of infancy and childhood in order to test certain basic and important hypotheses concerning the occurrence of neurological damage.

Methods Employed: Recognized damaged outcomes of pregnancy, especially mental retardation, have been studied and related to defined perinatal or postnatal events which are involved in the biological or psycho-sociological mechanism underlying the following hypotheses: (1) anoxia, (2) toxic influences on the brain, (3) metabolic influences, (4) trauma to the head, (5) infection of the brain, (6) dehydration of the child, (7) genetic or familial patterns, (8) socio-economic status, (9) prematurity, and (10) nutrition.

Current Status: Upon review the manuscript was criticized on the basis of possible bias inherent in incomplete data as presented. Since the data base is available on tape, another extended analysis is under way, comparing as well, the emerging findings from this retrospective study with those from the prospective COLR study when subjected to the same instrument. The validation of findings from the retrospective study would thus be possible.

The retrospective instrument designed for the collection and analysis of the Georgetown Study data has retrieved data from the COLR data base. The comparison of findings from these two sources of data shall be completed during FY77.

Honors and Awards: None

Publications: None

PERIOD COVERED:

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Public Health Implications Study of Perinatal Mortality in the
Collaborative Study (COLR) and in the Collaborative Study Cities.NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Z. A. Shakhshiri, M.D.

Special Assistant OPPE, NINCDS

OTHER: Mr. Ernest Harley

Systems Analyst EBRP, NICHHD

COOPERATING UNITS (if any)

LAB/BRANCH

OD-NINCDS

SECTION

Office of Program Planning and Evaluation

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

.05

PROFESSIONAL:

.05

OTHER:

.00

SUMMARY OF WORK (200 words or less - underline keywords)

Perinatal mortality of COLR children (single births 1959-1966) was compared by institution, race, sex, birth weight (b.w.), gestation length (g.l.) Regional trends appeared in a steady decline, except for 1962. Females, for both races, have lower b.w.; non-whites, for both sexes, have lower b.w., and shorter g.l. Perinatal mortality, as well as fetal mortality, is lower for non-whites at short g.l. and low b.w., and higher at long g.l. and high b.w. These data formed the basis of a term paper by Dr. G. Bartlett, in a Sociology course at Western Reserve.

Similar data are being analyzed from those COLR cities which supplied complete data base as requested. Comparison with COLR data variables (mortality, g.l., b.w.) will appear in a manuscript by end of FY'77. Additional studies are contemplated for later, on multiple births, controlling on factors known to effect mortality, g.l., b.w. (e.g.) parity, maternal age, prior pregnancies, legitimacy, etc.

Project Description:

Objectives: To evaluate fetal and infant mortality of the Collaborative Study population and of the cities from which that population is drawn, with the aim of comparing the two populations, city by city, and institution by institution, on mortality characteristics.

Methods Employed: In addition to the data previously available from the National Center for Health Statistics for perinatal events, detailed data on natality and perinatal mortality were sought for the study cities from either the state or city health departments, whichever had jurisdiction for these records. The data include figures for livebirths, stillbirths, and deaths under 24 hours, 1 day to 7 days, 8 days to 28 days and 1 month to 12 months, evaluated by birth weight, length of gestation, race and sex, and plurality for the years 1959 through 1966. Corresponding data were compiled by institution for the PRB study population. The state or city health departments furnished either completed tabulations or raw data to be tabulated.

Considerable effort has been expended to create a usable data file of the external data being obtained in connection with this study. The aim is to provide a file with more general utility than the limited scope of this study. When such a file is created, the information necessary to make use of the file will be made available to interested persons.

Current Status: There has been an unavoidable delay in the preparation of the manuscript describing the comparative trends of fetal, neonatal and infant mortalities in the COLR population and in the population of the cities where COLR institutions are located.

This manuscript shall be completed during FY77. The mortality trends of the COLR population are already in view, but are to be compared with those in the cities where the COLR institutions are located before the manuscript can be considered complete.

Honors and Awards: None

Publications: None

Annual Report
July 1, 1975 through June 30, 1976
Office of Scientific and Health Reports (OSHR)
Office of the Director
National Institute of Neurological and Communicative Disorders and Stroke)

NINCDS information, publications, press, and public affairs activities are centered in the Office of Scientific and Health Reports (OSHR). The Office is divided into three sections: Scientific Publications, Health Reports, and Public Inquiries.

The Office is responsible for advising the Director and executive staff on ways to enhance the Institute's public and professional image and on the effective interpretation and reporting of Institute-conducted and supported research findings. These findings are of interest and concern to many audiences, including Congress, the Department and other agencies of government, scientists, physicians, voluntary health agencies, and the general public. The Office initiates many programs, as well as responds to Congressional, Departmental, NIH, internal, and public requests.

This year and for the past 20 years, the OSHR has been responsible for producing many Special Reports on disease categories designated by the House Subcommittee on Appropriations. These state-of-the-art reports describe the disorder, present the Institute's program, review research advances of the past year including any new developments in therapy, and briefly project the outlook for the future. The eight Special Reports requested this year were: Stroke, Parkinson's Disease, Epilepsy, Cerebral Palsy, Spinal Cord Injury, Multiple Sclerosis, Neuromuscular Disorders, and Communicative Disorders.

Coordination of the Institute's program with the programs of some 60 private voluntary agencies and professional societies is a major function of the OSHR. This continued in Fiscal Year 1976 with the performance of many information services, including an annual directory of voluntary agencies; preparation of speeches and information materials as requested on specific disorders; meeting with representatives of voluntary agencies to provide advice; assisting with voluntary agency annual, scientific, and press meetings; and various other types of assistance.

Disease areas requiring special attention during the year were multiple sclerosis, amyotrophic lateral sclerosis, spinal cord injury and regeneration, hearing and speech disorders, epilepsy, autism and Huntington's disease.

Questions on implementation of the recommendations of the Multiple Sclerosis Commission were voluminous. A number of progress reports were printed and distributed, extensive amounts of correspondence from members of Congress and the public were handled, and information was provided on the inauguration of an MS clinical research program at the NIH Clinical Center.

A large volume of correspondence on amyotrophic lateral sclerosis related to patient admission in the ALS program at the Clinical Center, a controversial snake venom treatment for ALS in Florida, and the establishment of an ALS Foundation in California.

Spinal cord injury and regeneration research was the subject of widespread public interest this year, and considerable political pressure was brought to bear in order to increase this research. This was touched off by a trip made by Roger Frank, of Portland, Oregon to the U.S.S.R. for treatment of spinal cord injury, and capped by a visit to NIH and other medical centers by a team of Soviet neurosurgeons and neuroscientists. OSHR services responding to the surge of interest included arranging for a translation of one of the Soviet neurosurgeon's books; distributing a report on the current status of research in nerve regeneration; writing a lay summary of this report (to be published as a pamphlet), and handling large amounts of correspondence and telephone inquiries.

The Office assisted in the orderly phasing out of services formerly provided by the Information Center for Hearing and Speech at Johns Hopkins University. Bibliographies which had been compiled by the Center were duplicated and sent out to accommodate requests coming in after the Center had closed.

Epilepsy remained a subject of special interest throughout the year. Inquiries centered around the establishment of three comprehensive Epilepsy Programs, the release of a new anticonvulsant drug, and the establishment of an Epilepsy Commission.

A new emphasis on the problem of autism and the establishment of a Huntington's Disease Commission also required special attention.

In the day-to-day operation of the OSHR, the three sections produced publications, audiovisual materials, reports, press material, answers to public inquiries, and served as an information resource on the many disease categories within the Institute's purview.

The Office also provides services in connection with special events. These included the NIH Open House and the celebration of the Institute's 25th anniversary. Anniversary activities included preparing invitations; contracting for the printing of programs; conference, dinner, and transportation arrangements; taping and transcribing of lectures; and many other details.

Scientific Publications Section

The Scientific Publications Section produces and distributes publications, both for the general public and for the various scientific audiences. Publications production services are provided to various administrative units of the Institute, ad hoc committees preparing reports, and outside organizations in the neurological field when sufficient need is demonstrated. The services include planning, writing, editing, design layout, clearance, distribution, storage, and later revising and reprinting, according to demand. The Section works with the NIH Printing Unit, the Medical Arts and Photography Branch, and the Government Printing Office. It also serves as the Institute's supply center for publications.

The largest publishing project ever undertaken by the Institute was completed in December 1975. This was a three-volume, 2,000-page work reviewing the entire spectrum of neurological and communicative disorders, and was entitled "The Nervous System." It was published to celebrate the Institute's 25th anniversary, and contained chapters by over 160 distinguished scientists. The Section served as liaison between authors and editors in organizing the subject matter and in coordinating editorial changes. Distribution of some 300 sets to authors and various officials was also handled by the Section.

The Institute's Monograph Series has been expanded by one new publication and two others are presently in production. (Since 1965, 15 monographs have been published and 7 continue to be distributed). The latest monograph published, entitled The Scientific Basis of Spinal Manipulative Therapy (June 1976) comprised the proceedings of a large NIH conference on this subject, and has been distributed to some 2,000 medical schools, schools of osteopathy and chiropractic, and individuals. The new monographs in production present reports on the status of manpower in neurology and on manpower in the fields of audiology, speech pathology, and otorhinolaryngology.

The Section also assisted in editing the text and discussion of the Kroc Foundation Series, Vol. 5, GABA In Nervous System Function, based on the February 1975 GABA (gamma-aminobutyric acid) conference jointly sponsored by the Kroc Foundation and the NINCDS.

The Institute's highly popular Hope Through Research pamphlets and companion fact sheets on neurological and communicative disorders continued to be revised and reprinted as necessary. This year a new pamphlet on myasthenia gravis was published; another on Parkinson's disease is in press, and a third on stroke was in the planning stage as of June 1, 1976. Also, a new fact sheet on hydrocephalus was published, and two others are undergoing revision. There are now 16 leaflets in the Hope Through Research series and 5 fact sheets. These publications, three of which are also in Spanish, are widely circulated to private voluntary groups for distribution to patients and health workers and to students for use in health related classes and course work. A contract was negotiated with Supermarket Communications, Inc., to distribute 350,000 Hope Through Research leaflets in Fiscal 1977.

Bibliographies are also produced and distributed by the Section. These include the Cerebrovascular Bibliography, a quarterly selection of stroke references from Index Medicus; a 1,700-page Epilepsy Bibliography, covering the years from 1950 to 1975; and an annual bibliography of papers resulting from the Collaborative Study on Cerebral Palsy, Mental Retardation, and Other Neurological and Sensory Disorders of Infancy and Childhood.

Numerous other publication or revision projects were completed by the Section.

Public Inquiries Section

Staff members of this section spend a large part of their time responding to letters and phone inquiries. In the past year, more than 3,000 inquiries required individually written letters answering specific questions about neurological and communicative disorders. Many of these required research or coordination with the Office of the Director, intramural scientists and Institute grantees. Additional thousands of inquiries were answered with printed materials. In all, NINCDS responded to requests for 417,243 publications.

The OSHR interacts on a continuing basis with the 60 national voluntary health agencies and professional societies which make up the National Committee for Research in Neurological Disorders. This includes providing material on Institute research, programs, and personnel to the editor of the NCRND Newsletter, a publication of the National Committee.

Through an Exhibits Program, the OSHR brings the Institute's extramural activities to the attention of the professional societies and health agencies at national scientific meetings. Last year, a Program Exhibit was shown at the American Academy of Ophthalmology and Otolaryngology meeting in Dallas, the American Speech and Hearing Association meeting in Washington, D.C., the American Academy of Neurology meeting in Toronto, Canada, and the American Neurological Association meeting in San Francisco. All arrangements for scheduling and manning the exhibit were handled by the staff. These showings generated hundreds of requests for information about Institute programs.

This section also keeps abreast of Advisory Council meetings, plans the annual Council dinner, and updates a Council Directory. Additionally, the head of this section is the liaison person with the extramural area, is responsible for providing grantee information for use in Institute reports and publications, and writes annual special reports for Congress on cerebral palsy and spinal cord injury.

The OSHR also serves as the focal point for the Institute in discharging its responsibilities under the Freedom of Information Act. Under this Act, the Public Inquiries Section responded to 75 requests for summary minutes of the meetings and to 20 requests for substantive program material from the meetings. It also provided the Department with quarterly reports of this activity.

Health Reports Section

The Health Reports Section prepares and distributes Institute research and health reports (printed, filmed, and audio/video taped) to scientists, members of Congress, voluntary agencies and the public for both general information purposes and for continuing medical education.

The Section's audiovisual production/taping studio facility became fully operational at the beginning of FY 1976 in a converted laboratory in the Auburn Building. The facility's equipment includes new recorders which make precise videotape electronic editing possible and provide a production capability which can be upgraded to TV broadcast standards.

Using these and other facilities, the Section this year produced 18 additional lectures in the Neuromuscular Disease series, seven lectures given at a February NIH international conference on autism, and two special presentations about NINCDS for the NIH Open House.

There is a large demand for videotaped lectures on neurological subjects to meet continuing medical education programs of medical schools, medical centers, hospitals, private clinics and practitioners.

During the past year, the original 28 tapes in the neuromuscular series have been requested 2,573 times and are booked through August 1976. As of May 1, 210 users had booked the tapes 2,113 times for an average of 75 bookings per tape. The average showings per tape were 134 for a total of 3,639 showings. The average viewers per tape were 3,171, with total audience of 85,617 for all tapes.

The multiple sclerosis tapes in two months had been requested 202 times for an average of 22 bookings per tape scheduled through September 1976.

Section staff members taped patients at a conference on Dystonia Musculorum Deformans in New York City, and lectures in several other cities. Locally they (1) covered the press conference which launched The Commission for the Control of Epilepsy and its Consequences and that group's first meeting; (2) taped and distributed through the Epilepsy Foundation of America two TV public service announcements by the author of "I'm No Longer Living A Lie," an article which appeared in the February Good Housekeeping Magazine; (3) produced an audio tape of a February 1975 NINCDS-Kroc Foundation conference on gamma-aminobutyric acid, (GABA), and arranged its partially-NINCDS-subsidized distribution through Audio Digest, Inc., a non-profit subsidiary of the California Medical Association; (4) supported production personnel from ABC and a film company from CES Educational Films in their efforts to get interesting footage about NINCDS research; and (5) assisted Institute offices and other Institutes with their audiovisual projects.

Several new presentations are in production. These include one-hour programs on epidemiology methods, dystonia musculorum deformans, epilepsy, and lipid storage disease research.

Press activities are conducted and coordinated through the Health Reports Section. This year, to publicize the establishment of the Commission for the Control of Epilepsy and its Consequences, the Section prepared and distributed a press release to newspapers and the wire services, helped to organize a press conference at the National Press Building, arranged for a speaker, and videotaped and filmed the press conferences. Those interviewed included Senators and Congressman who were instrumental in developing legislation establishing the Commission.

Stories on Institute-supported or conducted research and announcements of personnel appointments and organizational changes were prepared by this Section. These appeared on a continuing basis in the NIH News and Features, the NIH Record, a number of daily newspapers, and national magazines.

The Section also continued to assist in gathering background information for news and features stories initiated by the press. Support provided to a New York Times Magazine reporter in setting up interviews with NINCDS experts on communicative disorders resulted in a widely disseminated story on noise and its effect on hearing.

Additionally, the Section helped to arrange and coordinate an interview with Dr. Wesley Bradley, Director, Communicative Disorders Research Program, with U.S. News and World Report, and later assisted in reviewing and revising the transcript. The resulting article, "How Modern Life Can Damage Your Hearing," is an excellent overview of disorders of communication which has been reprinted for use in responding to public inquiries on this subject.

The Section assisted Institute scientists and grantees in preparing for press interviews and presentations at the annual meeting of the Society for Neuroscience, and also participated in a science-writer's seminar which was attended by both journalists and scientists, including Institute grantees.

In the past year, the head of the Section served as coordinator of NINCDS-sponsored events in the NIH Open House. These included laboratory tours, video taped presentations of Institute-supported research, exhibits, and distribution of Institute publication. Arrangements for advance publicity were also handled by the Section.

The Section's audiovisual services are available to all NINCDS scientists. A system for requesting services, for deciding priorities and obtaining approvals has been established.

ANNUAL REPORT
For Period July 1, 1975 through June 30, 1976
Office of Biometry & Epidemiology
Office of the Director
National Institute of Neurological and
Communicative Disorders and Stroke

The Office of Biometry and Epidemiology (OBE) is established in the Office of the Director, NINCDS, to advise the Director and consult with the scientists in the Institute in the development of a program in biometry and epidemiology, and in the increasing application of these concepts and standards to programs of the NINCDS.

The Office of Biometry and Epidemiology is responsible for research and consultation in mathematics and mathematical statistics in the design, analysis, and interpretation of small and large-scale experimental investigations in neurology.

Ad-hoc descriptive and analytic epidemiologic investigations include studies of the occurrence of neurological disease in human population groups to establish base lines for the recognition of unusual patterns of disease occurrence and trends over time, provide clues to the etiology of disease, measure the social and economic impact of neurological disease on the U.S. population, permit the evaluation of treatment modalities, and facilitate the planning of programs and facilities for individuals with neurological disease.

It is responsible for the design, pretesting, and accomplishment of surveys to measure the incidence, prevalence, and economic costs of neurological disorders, distribution of treatment modalities, and associated measures of disability.

The Office of Biometry and Epidemiology provides systems analysis and computer programming for all program areas of NINCDS. It is aiding in the development of the NINCDS program information system.

The Office of Biometry and Epidemiology consists of the Office of the Chief, the Section on Epidemiology, the Section on Mathematical Statistics, the Section on Disease Statistics Surveys, and the Section on Systems Design and Data Processing.

The report on the projects and services performed by the Office is listed by major program area, and is followed by brief descriptions of Miscellaneous Activities, Publications, and Future Plans.

OFFICE OF THE DIRECTOR, NINCDS

Surveys

1. Surveys of the Incidence, Prevalence, and Costs of Neurological Disorders

- a. Head and Spinal Cord Injury
Contract No. N01-NS-4-2334
Contractor: Research Triangle Institute
Amount of Contract: \$757,124
Project Officer: Mr. William Weiss
- b. Multiple Sclerosis
Contract No. N01-NS-4-2335
Contractor: National Analysts
Amount of Contract: \$613,740
Project Officer: Mr. William Weiss
- c. Intracranial Neoplasms
Contract No. N01-NS-4-2336
Contractor: Westat, Inc.
Amount of Contract: \$661,431
Project Officer: Mr. William Weiss

The contractors for these surveys have completed their pilot studies. They were reviewed by the multi-disciplinary advisory committees associated with each survey, and by the Biometry and Epidemiology Advisory Committee of NINCDS. Recommendations for approval of the main phases of these surveys were made, subject to design changes based on the results of the pilot studies. These changes were incorporated in the design of the national samples, and OMB clearance documents for the three surveys were submitted. Two surveys have been approved, and are now in progress. The OMB clearance documents for the Multiple Sclerosis Survey are in review.

The three studies are expected to report their final results by December, 1976.

2. Survey of Incidence, Prevalence, and Costs of Stroke

Contract No. N01-NS-6-2333
Contractor: Westat, Inc.
Amount of Contract: \$460,565
Project Officer: Mr. Bernard H. Kroll

The contract for the survey of stroke costs, incidence and prevalence has been let. This survey, and the Intracranial Neoplasms Survey, are funded in part with DHEW set-aside evaluation funds.

The initial study design has been reviewed by a committee of experts and modifications to produce a final design have been completed and approved. Pretests of the proposed forms and procedures are underway. The various clearance documents have been approved.

3. Stroke Protocol in NCHS Survey

The National Center for Health Statistics has accepted the proposal from NINCDS that a stroke protocol be included in the 1977 Health Interview Survey. The primary objective of the stroke questionnaire is to provide an estimate of the proportion of stroke cases who do not receive care at a short-stay hospital. These data will supplement those collected from the national sample of short-stay hospitals.)

The final draft of the stroke protocol has been submitted and accepted by NCHS.

4. Design of the Epilepsy Prevalence Study

Principal Investigators: William Weiss, Bernard H. Kroll

New survey designs for identifying most epileptics are being explored. These will be used to measure the incidence and prevalence of epilepsy. The study will be conducted in close association with the National Epilepsy Foundation and the American Pharmaceutical Association.

5. Proposed System for the Collection of Comprehensive Chronic Disease Statistics

A proposal has been developed which outlines the elements of a system to provide annual trend data on the incidence (and, subsequently, prevalence) of a wide array of neurological (and other) disorders of patients treated at short-stay hospitals. The system has a potential for expansion so that patients with these disorders who receive ambulatory care by physicians, or who are cared for in nursing homes, but who receive no treatment at short-stay hospitals, can also be identified.

One possible approach is to redevelop some of the existing, recurrent surveys of the National Center for Health Statistics. The NCHS Hospital Discharge Survey, the Ambulatory Care Survey, and the Nursing Home Survey have the potential to be part of an integrated system for comprehensive disease statistics data.

Initial investigative work is being carried out to determine the extent of the revision in designs of the various NCHS surveys that would be required for the system, to solicit participation from other NIH Institutes with regard to disorders of their interest, and to recommend a pilot study involving joint NIH-NCHS sponsorship.

6. Invited Sessions on Surveys at the 1976 Meeting of the American Public Health Association

OBE has organized two invited sessions at the 1976 annual meeting of the APHA on the methodology and problems associated with national surveys of chronic disorders.

One session - Topics in Medical Provider Surveys - will report on the experiences of OBE staff and their contractors in the surveys of neurological disorders.

A second session will provide for a much-needed interchange between epidemiologists and statisticians in regard to the appropriate objectives of surveys of chronic disorders, their strengths and weaknesses, and the potentials which exist for meeting multiple objectives. The format will be that of a panel discussion.

EPIDEMIOLOGY

1. Multiple Sclerosis in the Shetland and Orkney Islands and Caithness, Scotland

Contract No. N01-NS-4-2321

Contractor: Massachusetts General Hospital

Amount of Contract: \$389,362

Project Officer: Dr. Bruce S. Schoenberg

Principal Investigators: Dr. David C. Poskanzer, Dr. Derek Roberts,
Dr. Paul Terasaki, and Dr. John Sever

The Shetland and Orkney Islands and Caithness, Scotland have the highest reported prevalence of multiple sclerosis anywhere in the world. The reasons for this are being examined. All patients with multiple sclerosis in the Shetland and Orkney Islands have been identified, and appropriate controls were selected for each patient. For such individuals (both patients and controls), as well as certain family members of these individuals, a questionnaire was administered and blood was drawn to measure the following: previous history of infection (confirmed by serology), dietary history, sanitation history, history of exposure to animals, occupational history, travel history, family pedigrees traced to 1776, history of allergic diatheses, blood group and genetic marker determinations (including HL-A, HL-B, and MLR determinations). Questionnaires and bloods have been collected for individuals on the Shetland and Orkney Islands. This material remains to be analyzed. It is expected that an additional 1-2 years will be required for complete analysis of these data and publication of appropriate reports.

The interview information has been entered onto code sheets designed by OBE. Complete data files have been prepared, edited and reviewed against original records. Preliminary tabulations for the Orkney Island have been completed and similar processing for the Shetland Island is under way. As laboratory and genetic determinations are completed, they will be submitted and added to the files for processing. The completed files will be used for extensive multivariate analysis.

2. Epidemiologic Study of Amyotrophic Lateral Sclerosis and Other Motor Neuron Diseases

Contract No. PH-43-64-44

Contractor: National Academy of Sciences

Amount of Contract: \$150,928

Project Officer: Dr. Bruce S. Schoenberg

Principal Investigators: Dr. Gilbert Beebe and Dr. John Kurtzke

The purpose of the study is to identify risk factors for the development of motor neuron disease and to determine the natural history of this group of diseases. The project is divided into three parts. In the first section of the investigation, World War II service medical records from 500 veterans who died of amyotrophic lateral sclerosis in the years 1963 through 1967 were examined to measure potential risk factors for the subsequent development of motor neuron disease. Appropriate controls were selected from service life insurance holders. The second part of the study involved an extensive interview survey of veterans currently hospitalized for motor neuron disease to again assess clues to etiology. Brain tumor patients were selected as matched controls. A final aspect of the study involves a follow-up of veterans discharged from VA hospitals with a diagnosis of amyotrophic lateral sclerosis to determine the natural history of the disease. It is expected that the first part of this project will be completed during the current fiscal year. Work on the remaining two sections is continuing.

3. Stroke Risk Factors in Panama

Contract No: N01-NS-3-2304

Contractor: University of Texas at Houston, School of Public Health

Amount of Contract: \$251,558

Project Officer: Dr. Bruce S. Schoenberg

Principal Investigators: Dr. Darwin Labarthe and Dr. Reuel Stallones

During the past year a study of stroke risk factors among various black populations in Panama was completed. Stroke and hypertension are known to be higher in blacks than whites. The purpose of this particular investigation was to examine "genetically distinct" black populations, each living in an urban and rural setting, to determine environmental and genetic factors in these conditions. Information on family history, life style, and cardiovascular disease was collected. In addition, blood pressure measurements, anthropometric measurements, electrocardiographic examinations, physical examinations, and laboratory examinations (including serum lipids and 20 genetic markers) were carried out. Despite numerous political difficulties, the group was able to collect what appeared to be good information on a group of rural English-speaking blacks and a group of rural Spanish-speaking blacks. These data are currently being analyzed and a final report will be prepared in this fiscal year.

4. Conference/Workshop on Neurological Epidemiology

Principal Investigator: Dr. Bruce S. Schoenberg

Plans have been initiated to conduct a two-day conference on neurological epidemiology. The purpose of the meeting will be to: (1) review the contributions of neurological epidemiology; (2) identify problems requiring further investigation and highlight areas likely to generate advances in

clinical neurology; and (3) recommend priorities for research in neuro-epidemiology. Invited participants will include distinguished investigators in this field and the meeting will be open to all individuals with an interest in the epidemiology of neurological diseases. The conference will form the basis for a publication serving to disseminate information provided at the meeting to all members of the neurological and neurosurgical community.

5. Prevalence of Major Neurological Disease in a Well-Defined Population of Southern Blacks and Whites

Principal Investigators: Drs. Armin Haerer and Robert Currier (Division of Neurology, Department of Medicine, University of Mississippi), and Dr. Bruce S. Schoenberg.

A number of preliminary investigations have indicated a rather marked difference in the prevalence of several neurological diseases in blacks as compared to whites. A possible artifact in such studies of neurological disease by race is that the black population may not be receiving the same medical care as the white population, and consequently neurological disease in blacks may be missed. In an attempt to circumvent this problem, an investigation is being designed to survey an entire county in Mississippi with a 40% black population. This county has a population of about 25,000 individuals, and was selected because of its population stability, cooperation of all existing medical personnel, and close physical proximity to the University of Mississippi. Using an initial questionnaire, each household within the county will be visited and inquiries made of each individual residing in the household as to the presence or absence of certain signs and symptoms. On the basis of this initial screening form, all individuals suspected of having neurological disease will be examined by Dr. Armin Haerer of the University of Mississippi. Well defined criteria for the diagnosis of major neurological disease will be developed. The survey will include cerebrovascular disease, convulsive disorders, mental retardation, dementia, cerebral palsy, and Parkinson's disease.

6. Descriptive Epidemiology of Primary Intracranial Neoplasms in Children

Principal Investigators: Dr. Bruce S. Schoenberg, Devera G. Schoenberg, Dr. Barbara W. Christine (Conn. State Department of Health) and Dr. Manuel R. Gomez (Department of Neurology, Mayo Clinic)

The descriptive epidemiology of primary intracranial neoplasms in children under age 15 was examined for the well-defined populations of Rochester, Minnesota, and the state of Connecticut. Information on incidence by age and incidence by histologic tumor type was obtained. These data were reviewed in relation to previous clinical series. It was possible to establish, for the first time, the incidence rate for primary intracranial neoplasms in children based on a well-defined population. Results from this study have been published (see list of publications).

7. Extracranial Malignancies Metastatic to Intracranial Neoplasms

Principal Investigators: Dr. Bruce S. Schoenberg, Dr. R. Jean Campbell (Department of Pathological Anatomy, Mayo Clinic) Dr. Albert Heck (Department of Neurology, University of Maryland), and Dr. R. Simon, Berkeley, Calif.

This study represents analysis of a series of cases in which an extracranial malignancy metastasized to a primary intracranial neoplasm. The pathological specimens are being carefully examined in hopes of providing clues to the etiology of this phenomenon. A paper is being prepared for publication.

8. Resolution of Reported Differences in the Incidence of Primary Intracranial Neoplasms

Principal Investigators: Dr. Bruce S. Schoenberg, Dr. Barbara W. Christine, (Connecticut State Department of Health) and Dr. Jack P. Whisnant (Department of Neurology, Mayo Clinic)

Age-specific incidence curves from the state of Connecticut reveal a small peak in childhood followed by a much taller, sharper peak with a maximum between ages 55 and 65. This also holds true for data collected from most large population-based registries. In contrast to this are the age-specific incidence curves for Rochester, Minnesota, which show a steady increase in incidence with increase in age. In addition, the reported incidence rate at any particular age for Rochester, Minnesota, is higher than comparable rates from other registries. The reasons for these discrepancies are currently being analyzed. A manuscript concerning the findings is being prepared for publication.

9. Descriptive Epidemiology of Primary Intracranial Neoplasms

Principal Investigators: Dr. Bruce S. Schoenberg, Dr. Barbara W. Christine (Connecticut State Department of Health) and Dr. Jack P. Whisnant (Department of Neurology, Mayo Clinic)

The descriptive epidemiology of primary intracranial neoplasms (including tumors of the pituitary gland) has been studied with regard to incidence, survival, and the association of these neoplasms with primary malignancies of other sites. A significant association was found between breast cancer and meningioma and this has been reported in the literature (see publications). A second paper describing incidence patterns by age, over time, and by histologic type is being prepared for publication. Data on primary intracranial neoplasms from the Third National Cancer Survey have been obtained from the National Cancer Institute and will be similarly analyzed. These data, however, include only those neoplasms specified as "malignant".

10. Cerebrovascular Disease in Children

Principal Investigators: Dr. Bruce S. Schoenberg, Devera G. Schoenberg, and Dr. James F. Mellinger (Department of Neurology, Mayo Clinic)

The Mayo Clinic experience with cerebrovascular disease in children was examined for the years 1965 through 1974. The medical record linkage system available for all medical facilities caring for the Rochester population makes it possible, for the first time, to establish an incidence rate for cerebrovascular disease in children. These data have been collected and are currently being analyzed. A paper based on these tabulations is scheduled for presentation at the April meeting of the American Academy of Neurology, and a manuscript is being prepared for publication.

11. Unusual Patterns of Cerebrovascular Disease

Principal Investigators: Dr. Bruce S. Schoenberg and Dr. Jack P. Whisnant (Department of Neurology, Mayo Clinic)

Unusual clinical patterns of cerebrovascular disease in the experience of the Mayo Clinic are being examined (e.g., the occurrence of more than 15-20 transient ischemic attacks/day). Such material is being critically evaluated in an attempt to link unusual clinical presentations with specific pathological lesions.

12. Study of Pre-existing Cardiac Disease and/or Hypertension as Risk Factors for Subsequent Completed Stroke and Transient Ischemic Attacks.

Principal Investigators: Dr. Bruce S. Schoenberg, Devera G. Schoenberg, and Dr. Jack P. Whisnant (Department of Neurology, Mayo Clinic)

This investigation is aimed at evaluating the effect of heart disease and hypertension as potentially treatable precursors of stroke and transient ischemic attacks. The objectives of the study are to determine the following: (1) the risk of stroke and transient ischemic attacks in individuals with heart disease and/or hypertension as compared to the risk in individuals without these conditions; (2) whether the existence of pre-existing heart disease and/or hypertension affects the type of stroke and whether it affects survival following stroke; and (3) whether there is a particular time interval following the onset of heart disease or hypertension during which an individual is at high risk for stroke. Data collection for this study has been ongoing since 1973, and it is estimated that an additional year will be required to complete the investigation.

13. The Association Between Myasthenia Gravis and Multiple Sclerosis

Principal Investigators: Dr. Bruce S. Schoenberg and Dr. Drake Duane
(Department of Neurology, Mayo Clinic)

There have been numerous reports of the association between myasthenia gravis and multiple sclerosis. The Mayo Clinic experience with these two diseases will be reviewed for cases of association, and this experience will be analyzed in an attempt to determine whether this association occurs more often than might be expected simply on the basis of chance occurrence.

14. Cerebrovascular Disease During Pregnancy and the Postpartum Period

Principal Investigators: Dr. Bruce S. Schoenberg and Dr. Burton Sandok
(Department of Neurology, Mayo Clinic)

This study will attempt to define the incidence and nature of cerebrovascular disease in the young female as well as the incidence of such events in the pregnant and non-pregnant population. This may provide us with information to better understand the increased risk of cerebrovascular events presumed to occur in association with the use of oral contraceptives. This investigation is just getting under way.

15. Study of an Increased Incidence of Neural Tube Defects in Infants Born in Somerset, Kentucky

Principal Investigators: Dr. Carol E. Anderson (Birth Defects Branch, Center for Disease Control) and Dr. Bruce S. Schoenberg

Investigations are currently being carried out to determine the frequency of neural tube defects in Somerset, Kentucky and its surrounding county. An increased incidence has been documented and ongoing studies are aimed at discovering the etiology of this unusual pattern of occurrence. A prospective study may be established to monitor the area's future experience with neural tube defects.

16. Investigation of Motor Neuron Diseases in Residents of Lehigh County, Pennsylvania

Principal Investigators: Dr. Matthew Zack (Cancer and Birth Defects Division, Bureau of Epidemiology) Dr. Lawrence Levitt, Neurologist, Allentown, Pennsylvania, and Dr. Bruce S. Schoenberg

In October, 1975, Dr. Levitt notified the Center for Disease Control about an apparently increased incidence of amyotrophic lateral sclerosis in Allentown and surrounding Lehigh County, Pennsylvania. Dr. Matthew Zack of the Center for Disease Control investigated this report and with the assistance of Dr. Schoenberg and Dr. Levitt developed criteria for the

epidemiological study of motor neuron diseases, investigated all potential cases, and established an incidence rate for these diseases in Lehigh County, Pennsylvania. A paper is currently being prepared concerning this investigation.

17. Investigation of a Reported High Incidence of Multiple Sclerosis Among Residents of Italian Extraction Living in Iron Mountain, Michigan

Principal Investigators: Dr. Bruce S. Schoenberg, and Dr. Carol E. Anderson, EIS Officer, Birth Defects Branch, Center for Disease Control, Atlanta, Georgia

On the basis of a report received from Dr. Jerry Chutkow, Mayo Clinic, of a number of individuals in the same family with the diagnosis of multiple sclerosis, an investigation was made of the multiple sclerosis experience of the Iron Mountain, Michigan community. Case records of all individuals from the community seen at the Mayo Clinic with a potential diagnosis of multiple sclerosis were reviewed and verified for the accuracy of the diagnosis. Initial efforts are underway to examine members of this community and to obtain blood specimens for HL-A, HL-B, and MLR determinations. There appears to be a definite excess of multiple sclerosis among community residents of Italian extraction. Several affected members of the community belong to a single family. This is quite unusual, and may provide significant clues, since residents of Italy are known to have a relatively low prevalence of multiple sclerosis.

18. Preparation of Video Tapes on the Principles of Neurological Epidemiology

Principal Investigators: Dr. Bruce S. Schoenberg, and Mr. Robert Finney

A series of video tapes concerning the principles of epidemiology is being prepared for use in medical schools, schools of public health, and training programs in clinical neurology and neurosurgery. These video tapes should serve as an effective means of increasing the sophistication of neurologists and neurosurgeons in the area of neurological epidemiology. The tapes, when prepared, would be loaned to appropriate institutions who request them. It is anticipated that an initial series of four such video tapes will be prepared during this fiscal year.

-STATISTICAL METHODOLOGY RESEARCH

1. Sensory Decision Theory for the Measurement of Pain

Principal Investigator: Dr. T. C. Chen

The Office of Biometry and Epidemiology has investigated the efficiency of a quantitative method currently proposed by some pain investigators for

the measurement of experimentally induced pain. This method, using a concept borrowed from sensory decision theory in psychophysics, claimed the capability of discriminating between the sensory and the psychological components in pain subjectively reported by patients. The accuracy of this claim has been debated among other investigators. The purpose of the study is to determine the validity of this method. A linear statistical model with probits was formulated and orthogonal contrasts were used for the investigation. The results indicate that under certain experimental conditions this method loses its ability to discriminate. Hence, the analytical results can be misinterpreted when this method is used in assessing the pain-relief effect of a drug or treatment. Future work will involve a computer simulation study to evaluate this finding.

2. Statistical Methodology for the Analysis of Neuronal Spike-Train Data

Principal Investigators: Drs. T.C. Chen and G.L. Yang and Mr. R. Richter

The Office of Biometry and Epidemiology has developed a research project for the study of statistical methodology for neuronal spike-train data analysis. The purpose of this study is (1) to investigate the nature of the current quantitative methods from an advanced stochastic process point of view, and (2) to develop a comprehensive statistical procedure which would incorporate more refined statistical techniques for analysing neuronal spike-train data. Discussions with Institute scientists have been held for advice on the validity of the concept of this study. References in the literature have been studied and a project proposal prepared. The University of Maryland Department of Mathematics will collaborate in the study.

3. Development of a National Pain Data Bank

Principal Investigator: Dr. T. C. Chen

The Office of Biometry and Epidemiology has developed a plan for the development of a national pain data bank, after studying its concept, function and potential utilization for research on pain. A report of this plan was prepared and presented at the First World Congress on Pain (1975) sponsored by the International Association for the Study of Pain. The plan has drawn interest and support from a large group of pain investigators. An essential preliminary is a nationwide effort toward standardizing the terminology that is used in the clinical diagnosis and treatment of pain, and developing a uniform list of clinical and laboratory information relating to pain. Pilot studies can be conducted within small groups of pain clinics to test various conceptual and technical problems that may exist in the establishment and operation of a pain data bank, without waiting for the completion of the above-mentioned task. The Office of Biometry and Epidemiology is considering the design of such studies with the support of IASP.

The Office of Biometry and Epidemiology organized an informational meeting on surveys of chronic pain. The Assistant Director of the University of

Washington Pain Clinic and an Assistant Professor of neuroepidemiology spoke on 'Chronic pain - a national health problem' and 'Headache, the scope of the problem'.

SYSTEMS ANALYSIS AND DATA PROCESSING

1. Budget Systems Design and Development

Principal Investigator: Sebastiano A. Sciabbarrasi

In order to develop an approach toward a program information system an intensive effort was made to review the current procedures for budget information reporting, to determine the sources and resources of budget data input, and to examine reporting requirements.

This effort has been substantially completed and a report has been written for the Program Planning and Evaluation Office for its use in developing the information systems within the Institute.

A draft of a contract project plan and request for proposals (RFP) was prepared. DHEW Set-Aside Evaluation Funds have been approved to support the development of the Program Information System. This will enable the Institute to obtain the professional assistance needed to develop the system specifications and some additional programming support for the entire system.

2. Annual Automatic Data Processing Report (ADPR)

Principal Investigators: Robert Richter,
Kenneth A. Elsner

The purpose of the annual study is to obtain and report all needs for automatic data processing (ADP) systems, software and services of the Branches and Laboratories of the Institute. It also estimates the financial requirements for these requests and presents them as an integrated package to higher authority for review and concurrence.

It permits an orderly development of ADP needs each year within the funds available for expenditure, consistent with the research plans of the Institute.

The system also includes reports on existing systems and software and meets the OMB requirements for reporting of machines, terminals and personal data systems.

3. Data Processing of Contract Files

Principal Investigators: Sylvia Edelstein, Loretta Cook

A contracts file is maintained for the use of the Contracts Officer, Extramural Activities. It is updated and modified on request by that Office. Monthly and demand reports are prepared.

4. Neuromed

Principal Investigators: Robert Richter, Alan Talbert

In prior years a computer system was developed to simplify the use of multivariate analysis methods by statisticians and data analysts in the Office.

During this year new programs were added and the documentation refined and improved. Plans for a seminar on its use and distribution of the program and methods to the statisticians of NIH have been made for sometime early in F.Y. 1977.

5. Investigation of Data Management Systems in Minicomputers

Principal Investigators: Robert Richter, Bernard H. Kroll

The advent of new technology and improvements in minicomputer memory size and speed have made it desirable to investigate the use of such devices for projects of the Institute. Design specifications have been submitted to various manufacturers and producers of software to determine the feasibility of such proposals. The results of this investigation, if favorable, may lead to the development of requests to obtain such software and/or equipment for specific NINCDS projects.

6. Morbidity and Mortality Journal Reference Resources

Principal Investigator: Naomi M. Hawkins

The Section has developed both computer and direct access to the National Library of Medicine files and resources (Offline-Medline and SDILINE) and prepares special reports for staff members in furtherance of their research, as requested. In addition regular weekly and monthly reports on specific disease categories are prepared for staff members.

DEVELOPMENTAL NEUROLOGY BRANCH

1. Cerebral Palsy Study

Principal Investigators: Drs. Karin B. Nelson and Jonas H. Ellenberg

The Office of Biometry and Epidemiology, in collaboration with Developmental Neurology Branch staff, has made considerable progress towards the production of a comprehensive monograph on cerebral palsy, based on the study data of the Collaborative Perinatal Project. The preliminary screen of antecedent obstetric variables and early clinical manifestations with regard to their association with cerebral palsy diagnoses has been completed. The statistical analysis of demographic factors (e.g., institution, race, socioeconomic status, etc.) and their impact on the incidence and risk of cerebral palsy is in the process of assessment. A study of the natural history of cerebral palsy from one to seven years of life has been compiled benefitting from the prospective nature of the Collaborative Perinatal Project. Part of this material was presented at the American Academy for Cerebral Palsy in September,

1975, and will be submitted for publication. Finally, the multivariate analysis of the obstetric, early clinical and demographic factors is in the planning stage. Extensive use will be made of the NEUROMED statistical package developed in the Office of Biometry and Epidemiology to facilitate the rapid execution of this complicated last phase of analysis. The completed manuscript for the monograph on cerebral palsy is expected by July 1977.

2. Convulsive Disorders Study

Principal Investigators: Drs. K. B. Nelson and J.H. Ellenberg

During the current fiscal year the convulsive disorders study overlapped with the risk screen and demographic analyses for the cerebral palsy study. Many of the listings of cases by special class groups and cross-tabulations are being accomplished in OBE. The significant factors associated with cerebral palsy and convulsive disorders differ as will the proposed multivariate analyses for the two studies. A cohort of some 1800 children in the Collaborative Perinatal Project who ever experienced febrile seizures is under study with regard to their risk for future afebrile seizures or other morbid outcomes. Segments of this material have been presented to the American Epilepsy Society (December 1975) and the American Epilepsy Foundation (January 1976), and have been submitted for publication. The completed manuscript for the monograph on convulsive disorders is expected by July 1977.

3. Birthweight-Gestation Study

Principal Investigators: Dr. J. Hardy and E. D. Mellits

The Office of Biometry and Epidemiology has participated in the preliminary analysis of the data for a study of prematurity based on the Collaborative Perinatal Project data. Graphs, tables and charts showing the relationship of birthweight and gestational age by race, sex, and survival status to be used as illustrations in the first phase of the report have been completed. Polynomial regressions and covariance tables have been analyzed to determine the extent of correlation between selected variables and birthweight and gestational age. These variables, along with others, will be used in phase two.

With the data analysis for the first phase completed, the initial write-up is in process. The Office of Biometry and Epidemiology is participating in the selection of variables and types of statistical analysis to be used in phase two. This phase is divided into three parts. First is an examination of the relationships between demographic and physical characteristics of the gravida and the dependent variables. Second, the relationships between the disease states and abnormal conditions affecting the gravida during pregnancy will be considered to identify those adversely affecting either, and/or both, birthweight and gestational age, and the other primary variables. Finally, a birthweight index will be developed.

4. "The First Year of Life", second volume continuing the presentation of the basic data of the CPP

Principal Investigators: Drs. J. Hardy and J. S. Drage, and E. Jackson

The Office of Biometry continued to participate in the preparation of the book, "The First Year of Life", presenting selected basic data from the Collaborative Perinatal Project. Current effort consists of preparation and review of text, and finalization of tables and charts.

5. Visual Abnormality Study

Principal Investigators: Dr. R. Feinberg and E. Jackson

Analyses of the relationship between a large number of demographic, obstetric, and pediatric variables with various visual abnormalities are under way. In addition, comparisons are being made of visual diagnoses at different ages. Further studies in additional depth will be made of those variables which appear important.

Case histories of blind children are being reviewed and a list of such children is being prepared.

6. Minimal Brain Dysfunction Study

Principal Investigators: Drs. P. Nichols and T. C. Chen

The Office of Biometry and Epidemiology provided extensive statistical support in the data analysis of this study. During the past year, the investigative team has completed a major statistical analysis toward developing new scale variables for the measurement of the manifestation of MBD children. Four scale variables representing school achievement, hyperactivity behavior, socio-emotional immaturity and minor neurological abnormality emerged from the correlation and factor analyses. The study did not indicate the existence of a unique MBD syndrome. The scores of these scale variables for each child in the cohort of study have been computed and included in the MBD file. Their distributions by sex and race were examined and compared. The preliminary results reveal some differences in the incidence of these symptoms among sex and race groups in the Collaborative Perinatal Project populations. Further work will be carried out to determine how these symptoms are associated in the MBD children. A large number of demographic, maternal, obstetric, and pediatric variables are currently being screened for their relationship with the MBD symptoms. A future study will relate these variables to various MBD groups.

EXTRAMURAL ACTIVITIES PROGRAM

1. Data Processing for the Otolaryngology Survey

Principal Investigators: Sylvia Edelstein, Barbara Nichols

The final tables for the analysis of this study were prepared during the year and the final report was published and submitted to the project officer by the contractor.

INTRAMURAL RESEARCH PROGRAM

1. L-Dopa - Bromocriptine Blind Study

Principal Investigators: Dr. R. Kartzinal, et. al., and D.A. Sadowsky

Twenty Parkinson patients were treated with a placebo (P); a transition (T) of no drugs, L-dopa, or sinemet; and bromocriptine (B). All patients were blind tested during all three (P, T, and B) periods on 23 variables (tremor, rigidity, speech, balance, dexterity, gait, posture, dyskinesia, etc.). Improvement and/or worsening of the patients under P, T, or B - and especially B (bromocriptine) was determined by analyzing the data on the 23 variables, plus three additional variables, an overall score, a mean tremor score, and a mean dyskinesia score. Improvement was determined by (1) using non-parametric statistics tests (Wilcoxin Matched-Pairs Signed-Ranks Tests, Kendall Rank Correlation Coefficients, Wilson's Distribution Free ANOVAS, etc.); (2) developing a measure of improvement or worsening expressed as percent of improvement or worsening; (3) analyzing and comparing the scores on the 23 variables with other variables (age, blood pressure, thalamotomy, dementia, duration of disease, etc.) by χ^2 tests, etc.; and (4) interpreting and graphing all results.

A preliminary report on this study was presented at the 28th Annual Meeting of the American Academy of Neurology, Toronto, Canada, April 30, 1976. A manuscript on this material is currently in preparation.

INFECTIOUS DISEASES BRANCH

1. Immunosuppression Study

Principal Investigators: Drs. W. T. London, J. H. Ellenberg, J. L. Sever and A. Palmer

This study involved the examination of the immune responses in severely nutritionally deprived rhesus monkeys. Three diet groups were defined (normal calories - normal protein, normal calories - low protein, low calories - low protein), after intensive examination of the necessary nutritional diet structure for the rhesus monkey. Monkeys were assigned to the two test groups and the control group and challenged with several different antigens (rubeola, rubella-mumps, DPT, VEE-flu) sequentially with sufficient time intervals between each administration to allow for washout of any prior infection effect. The test animal groups were then compared with control with regard to antibody response to the several antigens. The Office of Biometry and Epidemiology participation included design of the study, creation of a sophisticated computer control system for monitoring correct diet intake and weight gain, and analysis of results.

2. Herpesvirus Induction of Cervical Cancer in Cebus Monkeys

Principal Investigators: Drs. W. T. London, A. Nahmias (Emory University) and J. H. Ellenberg

Several studies have indicated that herpesvirus (HSV) type 2 may be etiologically related to human cervical cancer. This study is investigating the possible causal relationship between genital herpes and cervical cancer by attempting to induce cervical cancer in monkeys inoculated genitally with the virus. The statistical design of the study was produced by the Office of Biometry and Epidemiology in FY 72. Continual monitoring of the statistical aspects of the study has been required to make adjustments in the study design as necessary, due to differing rates of infection and reinfection in the Cebus model, and the excessive variability in the measures of antibody response and success of virus isolation. The study is expected to continue for at least another two years before analysis will begin.

3. Serological Study of Patient Infections and Pregnancy Outcome

Principal Investigators: Drs. J. L. Sever and J. H. Ellenberg

This study involves the determination of antibody responses to 11 infectious agents (mumps, rubella, herpesvirus, etc.) using serial sets of maternal sera which have been obtained prospectively from 5000 pregnant women with abnormal pregnancy outcomes and a similar number of matched controls. The general purpose of the study is to obtain information on the effect of maternal, serologically confirmed clinical and sub-clinical infections on the fetus and child. Staff of the Office of Biometry and Epidemiology have now completed the design of the statistical analysis of this study which is complicated by factors such as the definition of positive or negative antibody responses to specified antigens, where the blood samples were drawn at varying times during pregnancy. The computer programs for the analysis have been completed, and have been successfully tested on one of the abnormality groups. The analysis is expected to be completed by the end of the fiscal year.

4. Maternal Clinical Infections and Pregnancy Outcome

Principal Investigators: Drs. J. L. Sever and J. H. Ellenberg

In the Collaborative Perinatal Project, clinical infections were reported in approximately 5,000 of 58,282 cases studied. The children born of these pregnancies have been followed for up to 8 years. Data for all of the infections are being studied in terms of their relation to pregnancy outcome. The infected mothers will be matched with noninfected controls and comparisons made of rates of specified outcomes. This study was done in two parts; the first part examined associations of maternal clinical infection with outcomes in the children through the fourth year of their lives. The first part of the analysis has now been completed and the second part which deals with outcomes through the eighth year of life is expected to be completed

by the end of this fiscal year. Staff of the Office of Biometry and Epidemiology designed the statistical analysis of this study and worked in close liaison with staff of the Developmental Neurology Branch in developing complicated computer software necessary to implement the analysis.

5. Analysis of Cord IgM Levels from Births in the Collaborative Perinatal Project

Principal Investigators: Drs. J. L. Sever and J. H. Ellenberg

The general goals of this study are the examination of the association of cord IgM levels with specific mother (and/or child) abnormalities, and with etiologic characteristics of the cohort. The two phases of the study include the establishment of a normal IgM distribution, stratified for key relevant variables such as sex, race and maternal age and the comparison of morbidity in children with high IgM as contrasted with children with normal IgM. Questions of maternal leakage of high IgM, specific antigens generating the high IgM, low IgM etc., are also being considered. The Office of Biometry and Epidemiology participated in the design and analysis of this study, and we expect that it will continue into the next fiscal year.

6. Infections as a Cause of Infertility

Principal Investigators: Drs. Jones and J. L. Sever

This pilot study consisting of 76 patients and their controls was set up to consider the possible relation of infection with cytomegalovirus, herpes I and herpes II viruses and toxoplasma gondii to infertility. Clinical and laboratory evidence of infection with these agents was studied in subjects (patients attending the infertility clinic) and in controls (patients attending the obstetrical clinic at the same hospital). The analysis of the data was done using the Wilcoxon signed rank test and the paired t-test.

7. Analysis of Diabetic Mothers for Coxsackie B4 Antibody

Principal Investigators: Drs. J. L. Sever and D. A. Fuccillo

It has been reported that mice inoculated with Coxsackie B4 developed not only the previously recognized pancreatic disease of the noninsulin producing cells, but also late insulin dependent disease. Because of this report a study was initiated of 50 Collaborative Study women who have diabetes, together with matched controls for each patient. The sera from these women were tested for antibody to Coxsackie B4 virus by the neutralization method. The data was analyzed by the paired t-test.

8. Cellular Immunity in Multiple Sclerosis

Principal Investigator: Dr. D. A. Fuccillo

It has been reported that patients with multiple sclerosis (MS) lacked a specific cellular recognition for measles antigen. Preliminary results

on a small cohort of MS patients and controls were reported by Dr. Fuccillo in the April 26, 1975 Lancet. Subsequently, Dr. Fuccillo added more MS patients and controls to his cohort and the Office of Biometry and Epidemiology participated in the revision of the tabulations and statistical analyses of the new data set.

9. A Comparative Study of Antibody Levels found in Sera of Baltimore Inner City Children and Frederick County Children

Principal Investigators: Drs. J. Hardy and J. L. Sever

Approximately 1250 children ranging in age from 2 to 19 were included in this study. They were divided into 4 age groupings. Their sera were tested for antibody to herpes I, herpes II, rubella, CMV and toxoplasmosis. Comparisons between age groupings within a geographic area and between geographic areas were made by χ^2 tests. Tables, graphs and summaries of the statistical tests were provided to the principal investigators.

10. Systems Operation and Development of the Primate Information Retrieval System (PIRS)

Principal Investigators: Kenneth Elsner, Margaret Meadows, David Smith and Sebastiano A. Sciabarrasi

The System is used by the laboratory for colonies maintained by Dr. William London and his staff. It keeps a complete inventory of all monkeys in the laboratory colonies, their life histories, and a record of the procedures and studies for which these monkeys are used.

The reports supplied by the laboratory staff are processed by the Section, and edited and maintained in a data base. Reports are prepared on a regular basis and are used by laboratory staff to manage the colonies and develop studies.

11. Design and Implementation of Data Reports for the Cebus Monkey Study

Principal Investigators: Kenneth Elsner, Sebastiano A. Sciabarrasi

The laboratory data for the Cebus Monkey Study conducted by Dr. Palmer have been integrated into the PIRS data file. Output reports are in the final stages of development and testing prior to being made available to Dr. Palmer for use in his study.

SURGICAL NEUROLOGY BRANCH

1. Surgical Prophylaxis for Stroke

Principal Investigators: Drs. A.K. Ommaya and W. E. Lightfoote with D. A. Sadowsky among the co-investigators

A controlled four year study of 100-150 patients with transient ischemic attacks (TIA) is under way. These patients are to be treated with a

comprehensive medical regimen (CMR) (controls) and an STA (superficial temporal artery) - MCA (Middle cerebral artery) shunt surgery plus the CMR (diet, aspirin, persantin, and vitamins) (cases). To discover if the clinical improvement and reduction in TIA and stroke incidence is real, several statistical procedures must be followed - (1) set-up the protocol; (2) make this a truly controlled study; (3) randomize the order of patients receiving the CMR alone or the STA-MCA shunt plus CMR; (4) record neurological deficits by administering Clinical Quantitative Tests (CQT) before and at specified intervals after treatment; (5) assess the efficiency of the two treatments by Sequential Analysis; (6) collect, retrieve, process, analyze, and interpret all data arising from the study (the CQT's; the psychometric and social evaluations; data from electro-physiologic tests; isotope scans; tomography; angiography; and cerebral blood flow; etc.). At this time patients have been examined but have not been entered into the study since they did not meet all the physical requirements of the protocol.

2. Combination Therapy for Gliomas

Principal Investigators: Drs. A. K. Ommaya, J. H. Wood and D.A. Sadowsky

A retrospective investigation was undertaken of 143 patients, 125 of whom are available for a cerebral glioma study. These 125 glioma patients, during the past ten years, were tumor graded by the Kernohan system as astrocytomas Grade I, II, III, and IV, and treated with surgery; surgery and radiotherapy; or surgery, radiotherapy, and a variety of chemotherapies. In order to establish the limitations and advantages of these three types of therapy as a measure of survival and especially the quality of survival, the data were and will continue to be analyzed by use of modified life table techniques (for median survival times and for probabilities of survival or survival rates); ANOVAS; t-tests; regression and regression analyses; Kendall rank correlation coefficients and other nonparametric statistics (for determining the relationships of survival times to concomitant variables, etc.); graphs, etc.

A report on preliminary results of this study was presented at the National Cancer Institute Symposium on Modern Concepts in Brain Tumor Therapy: Laboratory and Clinical Investigation, Atlanta, Ga., February 26-28, 1976. A manuscript for this material is currently in preparation.

3. Neuron Counts and Sizes

Principal Investigator: Dr. R. Rajjoub

Statistical consultation was provided and analyses were performed on data obtained from five control and seven epileptic patients. The Purkinje's cell counts and surface measurements were tested for differences in counts and measurements between controls and epileptics, stimulated and non-stimulated in epileptics, and autopsied and stimulated in epileptics by use of ANOVAS and t-tests.

4. Craniotomy without Resection

Principal Investigators: Drs. J. M. Van Buren, C. Ajmone-Marsan, N. Mutsuga, and D. A. Sadowsky

Twenty-six epilepsy patients (over a 15 year period) who had undergone craniotomy without resection were studied to summarize their treatment and make comparisons with the treatment of patients with craniotomy with resection. The analyses and comparisons included: (1) frequency distributions, graphs, and basic statistics (\bar{X} 's and σ 's); (2) comparisons of fit frequencies with other variables (psychic change, ictus, seizure clusters, head injuries, etc.); (3) comparisons of pre- and postoperative data from a number of variables to determine their extent of association; (4) computing "incidence of seizure" rates (as developed by D. A. Sadowsky previously); and (5) comparisons of all these with the same information obtained for the patients with craniotomy with resections.

5. Cerebellar Stimulation - Seizure Study

Principal Investigator: Dr. J. H. Wood

Five epileptic patients have undergone a new type of surgery, namely, implantation of cerebellar stimulators. To test differences between GABA (gamma-aminobutyric acid) and NE (norepinephrine) in CSF, the patients received both cerebellar and caudate nucleus stimulation - no, unilateral, and bilateral stimulation; and pre-operative, post-operative, and post-stimulation stimulation. Relationships and differences between GABA and NE; no and unilateral stimulation; no and bilateral stimulation; pre-op. and post-op. stimulation; and post-stimulation and post op. stimulation were determined by correlations, t-tests, and ANOVAS.

DEVELOPMENTAL AND METABOLIC NEUROLOGY BRANCH

1. Measurement Data in Man

Principal Investigators: Dr. A. Dekaban and D. A. Sadowsky

This study is based on a group of 4760 (2784 males and 1976 females) patients, all of whom had measurements for 6 variables (age, sex, body weight in kg., height in meters, brain weight in kg., and 5 categories of diseases). These data were analyzed (using computers) by computations and evaluations of four sets of descriptive statistics - (1) means, variances, standard deviations, and standard errors of the means - height, body weight, and brain weight for both sexes for each year of age; (2) correlation coefficients - four indices as functions of body weight, height, and brain weight were obtained by sex and age and correlated with age to determine the extent and measurement of the relationship between these indices and age; (3) regression and regression analysis - to describe and label the above relationships, regression and regression analysis was used to fit the best fitting curves to these; and (4) graphic presentation - graphs were drawn to illustrate the

relationship between mean body weights, heights, and brain weights by age for both sexes; and the relationships between the four indices and age for both sexes. A manuscript for this material is currently in preparation.

LABORATORY OF NEUROPATHOLOGY AND NEUROANATOMICAL SCIENCES

1. Morphological Studies of CNS Demyelination and Remyelination in Model Systems

Principal Investigators: Drs. H. Webster and T. Tabira

Double blind tests were conducted by injecting various substances (CSF aliquot from 18 patients with or without demyelinating disease, rabbit sera, saline solution, etc.) into the optic nerves of tadpoles. The nerves were removed and the lesions created in them by the injected substances were counted. All experiments were conducted using randomized and double blind slide selection. The data from these lesion counts were analyzed and tested for differences between cases and controls, inoculated and non-inoculated sides, the various substances, etc. by use of the Kolmogorov-Smirnov Two-Sample Test, tests to determine whether the "count" data is compatible with a Poisson distribution, the Square Root Transformation, t-tests, ANOVAS, etc. These analyses enabled the investigators to test quantitatively for and differentiate demyelinating activity.

2. Demyelinating Activity of Cerebrospinal Fluid from Multiple Sclerosis

Principal Investigators: Drs. H. Webster and T. Tabira

This experiment is a new model, *in vivo*, system conducted as in Morphological Studies of CNS Demyelination and Remyelination in Model Systems reported elsewhere in this report, with the addition of refrigeration and heating of the CSF aliquots before injection, and the use of perineurial injections of the sera. The "count" data were analyzed and interpreted using the same method as reported elsewhere.

3. Mast Cells

Principal Investigator: Dr. J. Cammermeyer

In order to determine the number of mast cells in the area postrema, 12 animals were studied - six (3 males and 3 females) were treated with trypan blue, and six (3 males and 3 females) were used as controls. Cell counts, and light and heavy granulate cell counts were compared by ANOVAS and t-tests for differences between sex, weight, mast cell counts, light and heavy granulates, and treated and controls.

ELECTROENCEPHALOGRAPHY BRANCH

1. Potassium Kinetics in the Glumina Cream Focus of the Monkey

Principal Investigators: Drs. D. Lewis, N. Mutsuga and W. Schuette

Following a pilot study of six monkeys, a further study of 12 monkeys with drug induced seizures was conducted. In order to determine the relationship of fibrous astrocytes to potassium fall-off, the data were analyzed by use of regression, regression analyses, covariance analyses, ANOVAS, t-tests, graphing, etc.

OTHER ORGANIZATIONS

Department of Nutrition, School of Public Health,
University of North Carolina

1. Supplemental Food Program for Women, Infants and Children (WIC)

Consultant: William Weiss

OBE provided consultation leading to the final report on the evaluation of the data of the WIC program with regard to the potential benefits of the food supplements on the mothers and children.

Center for Health Studies, Research Triangle Institute

1. Targeted Study of Catastrophic Illness Addressing Spinal Injury

Consultant: William Weiss

Membership on a Technical Advisory Panel for this contract sponsored by the National Center for Health Services Research. This contract piggybacks on the NINCDS Head and Spinal Cord Injury Survey, and uses secondary source data as well.

The study's objectives include comparison of the effectiveness of alternative treatment systems.

Division of Cancer Control & Rehabilitation, NCI

1. Survey of the Cost of Cancer Care

Consultant: William Weiss

Membership on a committee to advise NCI on the development of a national probability sample survey to estimate costs of 12 major cancerous sites (excluding intracranial neoplasms). Initial steps include analysis of limited data of the Third National Cancer Survey, and multivariate analyses of costs by cancer site, mode of therapy, disease stage, and demographic sub-groups.

2. Division of Cancer Cause and Prevention, NCI

Consultant: Dr. Bruce S. Schoenberg

Provides technical merit review of all contract proposals, dealing with epidemiology and biostatistics, submitted to the Division of Cancer Control and Prevention of the National Cancer Institute, NIH.

Public Services Laboratory, Georgetown University

1. Study for Economic Cost of Diseases and Illness

Consultant: William Weiss

Technical merit review of progress of research contract on an historical study of costs of disease from 1900 to the year 2000.

Massachusetts General Hospital, Boston, Mass.

1. A Serological Study of Multiple Sclerosis Patients

Principal Investigator: Dr. D. Poskanzer

The Office of Biometry and Epidemiology has investigated a statistical problem in the estimation of rubeola and CMV titer levels for, and comparison between, multiple sclerosis and control groups, where the titer recording system includes both direct and indirect (rank order) measurements. The conventional two-sample tests in this case can not be appropriately applied. An alternative approach, which embraces the idea of data censoring and maximum likelihood estimation, has been derived and has been suggested for use in the analysis of unmatched data. The problem of analysis of matched data will be considered in a further study.

Washington Clinic, Washington, D.C.

1. A Screening Method for the Detection of Internal Carotid Occlusive Disease

Principal Investigators: Drs. W. Howell and T. C. Chen

A paper "Supraorbital Opacity Pulses During Carotid Compression: A Method for the Detection of Carotid Obstruction" in which an Office of Biometry and Epidemiology member is a co-investigator has been prepared and submitted to Stroke, a Journal of the American Heart Association, for publication.

Veterans Administration Hospital

1. A Biostatistical Study of Post-operative Back Syndrome

Principal Investigators: Drs. G. Mathews, T. C. Chen, et. al.

The Office of Biometry and Epidemiology has collaborated with the Veterans Administration Hospital, Washington, D. C., in planning and developing a multidisciplinary study of the postoperative back syndrome. The objective of this study is to evaluate the problem of unsuccessful operations of laminectomy and disclidectomy patients, to better define the postoperative back syndrome, and to derive some predictive criteria as to which features might indicate a predisposition to the postoperative back syndrome. The

biostatistical phases of the study include designing systems for the collection and computerization of preoperative, intra-operative and postoperative multidisciplinary data; investigation of the statistical nature and the correlational structures of these data; describing and discriminating between the cases of surgical success and failure; and deriving statistical criteria for predicting the probability of postoperative outcomes with pre- and intra-operative data.

Division of Resources Analysis, Office of the Director (NIH)

1. Revision of Normality Testing Program

Principal Investigators: Mr. A. J. Talbert

An existing program for normality testing (written by Alan J. Talbert in 1974) was radically revised, improved, and implemented in the form of a service program to be used by non-programmers. The emphasis is on ease of application. A literature study was made to determine the validity and limitations of equal expected frequency intervals, which are used in the chi-square test for normality in the program.

Clinical Center - NIH Medical Records Committee

1. Clinical Quantitative Tests

Principal Investigator: D. A. Sadowsky

A battery of Clinical Quantitative Tests has been formulated and presented before the NIH Medical Records Committee for review and approval. Final certification is under consideration, pending some revision and representation to the committee.

Mathematical Research Branch, NIAMDD

1. Nearest Neighbor Algorithms

Principal Investigator: Rosalind B. Marimont

The nearest neighbor problem may be stated as follows - given a set of points, x , in an s dimensional space, and a query point, q , to find the one or more of the x which are closest to q . Any metric may be used, but Euclidean distance is most common. The problem occurs in image processing, for $s=2$, and in many classification problems for $s > 2$. Drug screening schemes may involve high dimensionality. The straightforward distance computation can become quite expensive for large sets of points, and none of the algorithms that save computing time in a low dimensional space seem to work well in a high dimensional one.

A new method is being developed, and preliminary results are promising. The method has two features - first, the use of a lower dimensional subspace for a lower bound on distance, and second, rotation of the coordinate axes to ensure that the chosen subspace will be optimal for the nearest neighbor

problem. Simulations of 20 point library and query sets in a 10 dimensional space have been run in MLAB on the PDP10, and the dependence of the process on several parameters studied.

2. Non-Euclidean Graphs in Health Statistics

Principal Investigator: Rosalind B. Marimont

The problem is being studied in collaboration with Professor Max Woodbury of Duke University. A large sample of patients were rated on 5 factors relating to physical and emotional well-being. A cluster analysis showed a number of typical states and transitions between them, which could be studied by graphs. A property called parallelism was defined for pairs of transitions. It was found that in some cases, the definition led to anomalous results, in that "parallel" edges were incident on the same node. A graph with this property was called non-Euclidean. An algorithm has been devised and programmed to test for the non-Euclidean property, and a general characterization of the property is being sought. It is possible that a redefinition of "parallelism" may be necessary for the best analysis of the data.

This project is temporarily in abeyance because of the necessity for revision of some of the original data.

3. Methodology for Setting Numerical Goals and Timetables for Increasing the Number of Minorities and Women in Middle and Upper Grades

Principal Investigator: Rosalind B. Marimont

The project was assigned by the EEO officer, NIH. A system called FAIR (Feasible Allocation to Improve Representation) was devised. FAIR is based on the system embodied in the consent decree which settled the suit by the Equal Employment Opportunity Commission against AT&T. Data on accessions, separations, and promotions for the past 3 years were collected and smoothed to give reliable patterns. The FAIR system, which sets goals in terms of normal turnover rates and available manpower pools, was applied to these data, and computer simulations were run of three years of operations. Turnover rates at NIH are such that if goals are met, reasonable equality in GS grades 11-16 could be attained in from 5 to 11 years. A report embodying the methodology and results has been written, and is being studied by various segments of the NIH community, including the Acting Director, NIH, the Division of Personnel Management, and the EEO Council.

An abbreviated version has been accepted for publication by Public Personnel Management, (Journal of the International Personnel Management Association).

MISCELLANEOUS ACTIVITIES

1. Dr. J. H. Ellenberg

Regular reviewer for articles submitted to the statistical journal Technometrics.

2. Dr. Bruce S. Schoenberg

Abstractor in enzyme chemistry for Chemical Abstracts.

Production of a series of brief articles concerning eponyms in neurology for the Southern Medical Journal.

Book reviews in neurology for Science Books published by the American Association for the Advancement of Science.

Book Reviews in the history of neurology for the Journal of the History of Medicine and Allied Sciences.

Member, Board of Editors, Minnesota Medicine.

Reviewer of articles for the following journals: Journal of the American Medical Association, Journal of the National Cancer Institute, and Cancer.

Invited lecture:

Departments of Neurology and Neurosurgery
University of Mississippi, Jackson, Miss.
"The Epidemiology of Primary Intracranial Neoplasms"
September 1975

Presentation:

4th Pan American Congress on Neurology
Mexico City, Mexico
"The Incidence of Primary Nervous System Neoplasms:
International Comparisons"
October 1975

Presentation:

American Academy of Neurology
Toronto, Canada
"Cerebrovascular Disease in Infants and Children:
A Study of Incidence, Clinical Features, and Survival"
April 1976

Presentation:

Commissioned Officers Association Meeting
New Orleans, Louisiana
"The Association Between Breast Cancer
and Meningioma"
May 1976

Invited lecture:
Department of Medical Statistics and Epidemiology
Mayo Clinic, Rochester, Minnesota
"Case-Control Studies of Motor Neuron Disease"
May 1976

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- Schoenberg, B.S., and Schoenberg, D.G.: Of mice and men, of triumph and tragedy, of murine models of malignant disease. Surgery, Gynecology and Obstetrics 141: 933-937, 1975.
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Schoenberg, B.S., Mellinger, J.F., Schoenberg, D.G.: Cerebrovascular disease in infants and children: a study of incidence, clinical features, and survival. Neurology 26: 358, 1976.

Schoenberg, D.G., and Schoenberg, B.S.: Rounds with Dr. Thomas Willis. Accepted for publication by the Southern Medical Journal.

FUTURE PLANS

With the expected recruitment, near the end of this fiscal year, of two survey statisticians, plans for the next fiscal year include a start on the analysis of the data of the intracranial neoplasms, multiple sclerosis, and head and spinal cord injury surveys.

Pilot studies and workshops in anticipation of a major epilepsy survey are planned. A new approach has been formulated for identifying a large proportion of epilepsy patients, and this approach will be considered for implementation.

If a cooperative relationship can be developed between NIH and NCHS, we propose to develop a pilot study to test the feasibility of obtaining annual trend data on the incidence and prevalence (subsequently) of a wide array of neurological diseases, using redesigned, recurrent surveys of NCHS.

OBE, in cooperation with the Communicative Disorders Program, plans to develop a pilot study of speech and hearing disorders of elementary school children in a school system in Maryland.

There are a variety of neurological disorders with high relative frequency, for which no adequate population-based data are available. A national sample of the general population will be proposed to determine epidemiologic patterns of headache by type, and to elucidate features of the clinical history and examination which may be useful determinants in prognosis and treatment.

There is a paucity of information on the relative frequency of dementia in the elderly, and a study of this condition, its course and outcome, in a probability-based sample of the general population of elderly individuals will be proposed, in collaboration with the National Institute on Aging.

Similarly, a study of the relative frequency of lumbar and cervical radiculopathy will be proposed.

ANNUAL REPORT
Associate Director's Report
July 1, 1975 through June 30, 1976
Extramural Activities
National Institute of Neurological
and Communicative Disorders and Stroke

The Extramural Activities Program (EAP), NINCDS, was organized in July 1975, to serve as the Institute coordination and focal point for science administration, fiscal management and administrative management of its grant, fellowship and research contract activities. The structure of EAP includes units responsible for extramural committee management, data reporting and analysis, scientific merit review, contract management, grants management and program support services including processing; executive focal points also have been established for the research grant programs, the research contract programs and the manpower development programs. Fiscal Year 1976 was characterized by the establishment of these organizational units, the definition of responsibilities, the development of operational patterns and the conduct of Institute extramural activities; this latter point must be emphasized for as these important organizational changes were being developed and implemented, the extramural program activities of the Institute were in full operation and had to be maintained at high levels of effectiveness and efficiency. I am pleased to report that both the required organizational changes were able to be implemented and ongoing program activities continued with a minimum of confusion or disruption. Issues that require further attention include functional elaboration of the responsibilities of program area staff and EAP staff, relationships and relative responsibilities of the Institute Program Planning Office and the EAP Data and Report Unit, and the relative roles of EAP staff and program area staffs to the activity of the National Advisory Council.

A major physical move of importance and consequence was accomplished in March, 1976. This move placed all EAP personnel and all program area personnel in the same building. As with any physical relocation, the move is a mixture of blessings and horrors. With time and the development of new work patterns, most problems will be resolved. A small number of renovations are critically required; improvement of poor lighting resources now being justified by antediluvian lighting standards need attention, and improved messenger services between the Federal Building, the Westwood Building, and the NIH campus require development.

As indicated in the reports of the Extramural Program Directors and the EAP Program Coordinators (EAP Deputy and Assistant Directors), the delay in the FY 1976 appropriation and the uncertainties of the probable level of the appropriation delayed the implementation of Institute plans for the first 8 months of the fiscal year. During this period, relying upon innovative management techniques and excellent rapport with the scientific community, staff focused on maintaining the core research program and the development of alternatives for program implementation at an appropriate time. Conceptual approaches to program implementation at a flat level budget are being developed and pilot activities initiated to pre-test these concepts.

In summary, the development of the EAP organization and its physical relocation both occurred in Fiscal Year 1976. Several tangible problems of space and resources require solution; a number of less tangible problems involving the working relationships among staff require more precise definition and focused attention. The tardiness of appropriation decisions and the relative insecurity of appropriation and personnel resources have delayed the initiation of needed program implementation and the maintenance of ongoing programs. However, by relying upon both the staff's excellent rapport with the neuroscience and communicative science communities and the use of innovative administrative techniques, the core program was continued with a minimum of disruption or uncertainty.

ANNUAL REPORT
July 1, 1975 through June 30, 1976

EXTRAMURAL ACTIVITIES (RESEARCH GRANTS)
NATIONAL INSTITUTE OF NEUROLOGICAL AND COMMUNICATIVE DISORDERS AND STROKE

This portion of the NINCDS Annual Report is intended as an overall summary of administrative, logistical and personnel problems and developments as they pertain to the Extramural Activities Program, particularly research grants. For purposes of cohesiveness, however, other activities (training awards, contracts, etc.) are mentioned although they are discussed in more detail elsewhere.

The research grant, contract and training programs of the NINCDS are focussed on the identification, stimulation, and support of essential research problems aimed at the improved diagnosis, treatment, and prevention of disorders of the nervous system, the neuromuscular apparatus, the ear, and human communication. They include disorders of the young (cerebral palsy, epilepsy, learning disabilities) of adulthood (head and spinal cord injury, multiple sclerosis, brain tumors) and of the aged (stroke, parkinsonism, otosclerosis). The administrative instruments used to accomplish these purposes include research projects, research program projects, clinical research centers, outpatient clinical research projects, specialized research centers, research career awards, research career development awards, teacher-investigator awards, institutional research fellowship awards, individual research fellowship awards and contracts.

The following Table shows the number of research grant applications considered by the Council at its June meetings in recent years.

<u>JUNE '70</u>	<u>JUNE '71</u>	<u>JUNE '72</u>	<u>JUNE '73</u>	<u>JUNE '74</u>	<u>JUNE '75</u>	<u>SEPT. '76*</u>
339	352	398	467	428	481	493

*Comparable to the June meetings on the basis of the new fiscal year.

The number of applications has increased more than 45 percent. This is attributed primarily to the effectiveness of the research training programs of the Institute in which the output of fully trained investigators has only in recent years reached its full potential. It is tempting to suggest that the leveling off in the number of requests since FY 1973 is due to the proposed phasing out of the training program in 1973. Another factor may be that, because available funds have in the past permitted the funding of such a small proportion of approved proposals, many investigators have been discouraged from applying.

The following Table shows the number of research grants awarded and the total amounts of funds expended (in millions) each year for the past nine years.

	<u>FY '68</u>	<u>FY '69</u>	<u>FY '70</u>	<u>FY '71</u>	<u>FY '72</u>
NUMBER	1,780	1,798	1,267	1,256	1,280
DOLLARS	\$65.1	\$67.8	\$48.8	\$48.8	\$64.2

	<u>FY '73</u>	<u>FY '74</u>	<u>FY '75</u>	<u>FY '76</u>
NUMBER	1,056*	1,065	1,221	1,200
DOLLARS	\$62.4*	\$70.0	\$86.6	\$90.4

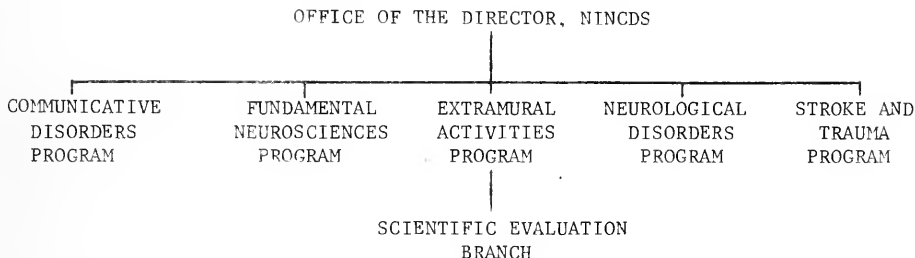
*Exclusive of impounded funds.

The largest change was in FY '70 when all research on vision was removed from this Institute to form the National Eye Institute. At that time the number of grants decreased by 29% and the amount of funds by 28%. Until FY '72 the number of projects supported has been maintained at approximately the same level each year despite a marked increase in the cost of all aspects of doing research. This was made possible in part by modest increases in the amounts of funds available, but primarily by the fact that beginning in FY '70 every research grant, competing and committed, was subject to a negotiated reduction. All grants were negotiated downward by an amount which hopefully would still allow the work to proceed, although productivity doubtless was reduced because costs increased each year. These reductions, in effect, made additional funds available for competing proposals in the amounts of \$7.0 million in FY '70, \$5.0 million in FY '71 and \$3.0 in FY '72. FY '72 was the last year in which such reductions were made because the National Institutes of Health decided that it was preferable to support fewer and better projects adequately than to support more projects with insufficient funds to do the work properly. The number of projects supported immediately decreased by 16%. It should be emphasized, however, that even after reducing each grant, funds were sufficient to support only the better half of approved proposals. Some increase in funds in FY '75 and FY '76 made it possible to bring the numbers of grants back almost to the number awarded in FY '70 after all of the projects on vision were transferred to the National Eye Institute. Perhaps it should be mentioned that for many years the approval rate for grant applications by the Initial Review Groups was almost exactly 50%. During the past 3-4 years this has increased to about 65%. We believe this is due to the fact that an increasing number of disapproved applications and many approved, but unfunded proposals, with the help of comments from the Initial Review Groups are being resubmitted in an improved form. Funds available this year permit supporting only about 25% of approved proposals.

For many years the Extramural Program was organized on the basis of a Research Grants Branch and a Training Grants and Awards Branch. During FY '74 the activities were reorganized along more functional programmatic lines consisting of a Regular Programs Branch and a Special Programs Branch. The primary responsibility of the former was the planning, development and operation of the investigator-initiated research grant programs. The primary

responsibility of the Special Programs Branch was the planning, development and operation of the targeted grant programs including program projects, clinical research centers, special projects, training programs and manpower studies.

During the past year (FY '76) the entire extramural effort of the Institute has been reorganized and the complete grants and training activities have been moved (March, 1976) from the Westwood Building to the Federal Building. The reorganization into four scientific program areas and one administrative and supervisory Extramural Activities Program is illustrated below:



During the past eight months Program Directors have been appointed for each of the four scientific program areas. Members of the professional and secretarial staff of the former Regular Programs Branch and the Special Programs Branch have been distributed among the four program areas and have been combined with staff from the former Collaborative and Field Research Program who worked primarily with contracts. Thus, the Director of each Program theoretically may use either grants or contracts within wide limits to accomplish the objectives of his Program. The question of whether research training awards are to be fully interdigitated into this arrangement is still under consideration. They have been coded by Program Area so they may be included as part of the total effort of the appropriate Program. However, the management and administration of research training awards is so different from that for all other types of awards and the amounts of funds involved are presently so modest, that it seems doubtful whether it would be advantageous to fully integrate them with grants and contracts from the standpoint of grant administration and management.

The Extramural Activities Program provides supervisory, coordinating and service functions for the other Programs as well as for extramural activities in general. The top staff consist of:

Director
Deputy Director (Research Grants)
Assistant Director for Manpower Programs
Assistant Director for Contract Research

The responsibilities of these people are apparent from their titles in that they carry out an overall coordinating and supervisory function in regard

to the implementation of recommendations of the NANCDS Council and Contract Advisory Committees, and the processing and issuance of proposals and awards in their respective areas. The Director, in consultation with the Director NINCDS, in addition works closely with the other Program Directors on questions of policy, distribution of funds, etc. Each of these people have a summary of their activities elsewhere in this Report.

The Extramural Activities Program also includes a Grants Management Branch, a Contracts Management Branch and an Office of Data Analysis and Reports, each of which has its own summary of activities elsewhere in this Report.

Also included is a Scientific Evaluation Branch with the responsibility for the initial review of all grant and contract proposals which are not reviewed by Study Sections of the Division of Research Grants, an activity of the Office of the Director, NIH. This Branch is presently composed of five professional personnel and supporting staff including the Executive Secretaries of the Communicative Disorders Program Project Committee, the Neurological Disorders Program Project Committee A and the Neurological Disorders Program Project Committee B. The establishment of this Branch makes it possible, in agreement with NIH policy, to have a true dual review system for grant applications and contract proposals in which the initial review is carried out by Committees within the Institute. Previously the Executive Secretaries usually administered and managed grants which were reviewed by their own Committees. This responsibility has now been taken over by the staff of the Program to which the proposal is assigned. This Branch also has a summary of its activities elsewhere in this Report.

Last, but not least, is the Office of Program Support and Awards (grants and contract processing). This group prepares, coordinates and assembles all of the material for the final review of all grants and contracts. It sees that copies of proposals are distributed to appropriate staff members and it assembles 61 sets of books for each National Advisory Council meeting, each set consisting of three books totalling some 500 pages of initial review group recommendations. It also assembles similar books of material for the Council Planning Subcommittee which meets about two weeks before each Council meeting.

The rationale for the reorganization is that the Director of each Scientific Program should have available all of the mechanisms (grants, contracts, training awards) and he should use them more or less at his discretion to best accomplish the objectives of his Program. It is further felt that the Program Director, in consultation with the Advisory Committee which is being established for each Program, should exert a fair degree of control and direction on the program to best meet its specific objectives. It was decided that this arrangement would function most effectively if the grants activities and the contract activities were housed together. Therefore, the grants staff were moved from the Westwood Building to the Federal Building in March, 1976.

Reservations on the part of the professional staff as to whether this arrangement would in fact result in more effective research programs were expressed in some detail in last year's Report. The main one was that science

does not develop in a hit and miss fashion. New areas develop only after the original basic research has been done. The scientific community most likely to sense these opportunities, and therefore, investigator-initiated research is most likely to develop new, productive areas of research. If this thesis is accepted, any appreciable degree of direction or control on a program may actually be disadvantageous except for highly applied aspects in which it is clear that a specific job needs doing. Contracts were intended to serve rather specific functions. An appreciable degree of interchangeability between grants and contracts may work to the detriment of both programs.

The problems which are arising are exactly those which have been faced by all other Institutes organized in this way. The fundamental problem is to avoid having the extramural effort of the Institute break up into four separate and distinct programs, each going its independent way scientifically and administratively. It is well known that research in one area is frequently applicable to problems in another area. Therefore, it is essential that the Programs be interrelated through staff discussions and adequate liaison.

This problem can be resolved to some extent by avoiding the allocation of specific amounts of funds to each Program. This Institute and several other Institutes have done this to a large extent for grant funds. In general rather specific allocations of contract funds are made to each Program. In the case of grants, the Program Directors have agreed that the best proposals above a certain priority cut-off should be supported regardless of what their relevance to a particular Program appears to be at the moment. An effort is made to reserve a modest amount of funds which the Program Directors may use to support other approved projects which they feel are especially important to their Program. Since the needs and the opportunities of a specific Program will vary from year to year, the advance allocation of specific amounts of funds to each Program will only result in feast or famine rather than in the effective development of new research fields because important unanticipated opportunities that arise in the various Program Areas cannot be predicted.

In summary the reorganization and the move were based on the philosophy that:

1. A Program Director should exercise an appreciable degree of direction and control of his Program.
2. This can be best accomplished by each Program Director having available at least the two main mechanisms of project support (grants and contracts) which should be utilized more or less interchangeably to attain the objectives of the Program.
3. The joint utilization of grants and contracts would be most effective if the respective staffs are housed in close proximity.

The first two points have been commented on above. The group that probably is most disadvantaged by the move to the Federal Building is the Office of Program Support and Awards (grant and contract processing). This group obtains thousands of copies of applications and summary statements from the Division of Research Grants in the Westwood Building for each round of

meetings. Particularly in regard to summary statements time is of the essence because of the time required to prepare them by the Executive Secretaries after the Study Section meeting and the urgency of receiving them as soon as possible in order to include them in the books to be sent to the Council members as far as possible in advance of the Council meeting. The professional staff too, find the present arrangement to be of considerable inconvenience since they also frequently need to obtain special items from the Division of Research Grants or have special problems for which a face to face discussion with members of the Division of Research Grants staff (sometimes two or three) would be much more effective than telephone conversations. There have been a number of instances in which essential material was greatly delayed or was delivered to the wrong location. As far as can be determined, this is the only Institute in which arrangements were specifically made to house the grants staff in close proximity to the contract staff. Only time will tell whether this will increase program effectiveness enough to make up for the inconvenience and frustration of being at a distance of several miles from the Division of Research Grants.

There is little doubt that the reorganization has had a deleterious effect on the morale of the professional staff who were formerly associated only with research grants. These people were appointed, most of them many years ago, as scientist administrators. They were expected to be or to become experts in grant administration and management. They were not expected to have more than a smattering of knowledge about the many scientific areas represented by the grants for which they were responsible. Now they are part of the staff of a Program in which the ultimate emphasis will be appreciable direction and control of the research to be supported for which an expert knowledge of the scientific fields is highly desirable if not required. Naturally, they are concerned about their long range future as new staff are brought in just out of the research laboratory and with an in-depth knowledge of the field which they represent.

As a matter of record Table I is attached which shows the number of awards and the amounts of funds expended for each type of award within each Program Area.

TABLE I

Number of Awards and Dollars Expended by Program Area and Type of Award

TYPE OF AWARD	PROGRAM AREAS					ST	TOTAL
	CD	FN	ND				
Research Grants	No. 219 \$11.154	No. 289 \$14.830	No. 488 \$27.160	No. 125 \$11.741	No. 1121		\$59.885
Program Projects and Clinical Centers	No. 16 \$ 6.027	No. 11 \$ 3.255	No. 27 \$11.273	No. 25 \$9.536	No. 79		\$30.091
Contracts	No. 16 \$.634	No. 15 \$ 1.591	No. 69 \$ 7.441	No. 24 \$1.169	No. 124		\$10.835
Old Training Grants	No. 13 \$.753	No. 4 \$.249	No. 31 \$ 2.097	No. 4 \$.225	No. 52		\$ 3.324
New Training Grants	No. 5 \$.293	No. - \$ -	No. 7 \$.685	No. - \$ -	No. 12		\$.978
New Fellowships	No. 31 \$.419	No. 30 \$.405	No. 180 \$ 2.430	No. - \$ -	No. 241		\$ 3.254
Teacher-Investigator Awards	No. 3 \$.067	No. 1 \$.020	No. 13 \$.304	No. 3 \$.077	No. 20		\$.468
Research Career Development Awards	No. 6 \$.139	No. 17 \$.366	No. 53 \$ 1.140	No. 2 \$.045	No. 78		\$ 1.690
Research Career Awards	No. 2 \$.063	No. - \$ -	No. 5 \$.157	No. 1 \$.031	No. 8		\$.251
TOTALS	No. 311 \$19.549	No. 367 \$20.716	No. 873 \$52.687	No. 184 \$17.824	No. 1735		\$110.776

(Dollars in millions)

July 1, 1975 - June 30, 1976

ANNUAL REPORT
July 1, 1975 - June 30, 1976
Contract Research Programs
Extramural Activities
National Institute of Neurological and Communicative
Disorders and Stroke
National Institutes of Health

The contract research programs of the Institute are primarily concerned with practical use of knowledge and are designed to meet recognized needs in cases where NINCDS has identified specific research problems requiring central direction, control, and management. The sharp overall upsurge in contract research supported by the NIH has not been reflected in the NINCDS where the contract budget has remained at a level of approximately seven to eight percent of the Institute's budget for the last half-dozen years. This evenhanded approach has allowed the NINCDS to take advantage of promising research leads not being exploited under the grant mechanism without jeopardizing its primary commitment to investigator-initiated grant-supported research.

Since July 15, 1975, the Institute has been operating under its reorganization by program areas. Contract programs are now served by four separate groups in the Extramural Activities Program. Contract solicitation, negotiation, and administration are carried out by the Contracts Management Branch under the direction of the Contracting Officer. Technical merit review is the responsibility of the Scientific Evaluation Branch which utilizes both ad hoc review groups and established committees in carrying out its mandate. Processing of RFP requests and receipt of contract proposals is part of the work of the Office of Grants and Contracts Processing. The Extramural Data Systems Section is beginning the task of providing support for the contract information system. Integration of the work of these groups with each other and with the program areas has proceeded smoothly and effectively.

The reorganization served as the impetus for drafting a Policies and Procedures document for contract research project initiation. After several drafts and much discussion with the Program Directors and the Office of the Director, NINCDS, the new policies became effective April 1, 1976. The guidelines cover both contract solicitations and unsolicited proposals.

Elsewhere in this report, projected contract numbers and expenditures are given with all contracts divided into four areas--communicative disorders, fundamental neurosciences, neurological disorders, and stroke and trauma. Another, and administratively more useful, way of looking at the same data is to divide the contract program into seven parts:

	<u>Number of Contracts and Agreements*</u>	<u>Dollars**</u>
Intramural Research Program	26	2,697,000
Biometry and Epidemiology Branch, OD	7	464,000
Communicative Disorders Program (Extramural)	16	634,000
Fundamental Neurosciences Program (Extramural)	14	1,543,000
Neurological Disorders Program (Extramural)	44	4,596,000
Stroke and Neural Trauma Program (Extramural)	20	901,000
Extramural Activities Program	<u>1</u>	<u>-0-</u>
TOTAL	128	\$10,835,000

*Includes contracts administered by NINCDS but funded by other Institutes.

**NINCDS funds only.

This breakout emphasizes the administrative units involved: the five Extramural Program areas, Intramural Research, and the Office of the Director, NINCDS.

One significant trend deserves special mention; a rapidly increasing major fraction of the NINCDS contract budget is being utilized to further the development of comprehensive centers aimed at alleviating two very important neurological problems--epilepsy and stroke. The potential impact of this expenditure, relative to the funds available to directed research for the entire mission of the Institute, deserves special attention.

Annual Report
July 1, 1975 through June 30, 1976
Manpower Programs

National Institute of Neurological and Communicative Disorders and Stroke

As the Institute entered FY '76, we continued to phase out support for the old training grant program and the Postdoctoral Research Fellowship Program which was authorized under the Weinberger authority. However, we were intimately involved in four other training activities -- the Institutional and Individual National Research Service Award Programs, the NINCDS Teacher-Investigator Program, and the Research Career Development Award Program. Although a summary of the awards made in each of these programs appears in Table 1, an explanation of the data is in order. The old training grants data show 52 awards which were made to honor the final periods of recommended support for this program. The figures for new training grants (Institutional National Research Service Awards) show the amount awarded to support in the -02 year the initial twelve awards made in this program. The figures do not indicate that any new Institutional NRS awards will be made from FY '76 funds. The figures for new fellowships (Individual National Research Service Awards) represent a combination of 104 continuation awards and 137 new awards. Fifteen of the new awards were made following the November Council meeting and the remainder we anticipate making following the June Council meeting. However, the number of new individual awards will be reduced if any new institutional applications are funded. Finally, the Table does not include 14 Postdoctoral Research Fellowship continuation awards made under the authority of the old Weinberger Program which is in a phase-out status.

Old Training Grant Program

Two year awards were made to 52 grantees and this was for their final period of recommended support. Under this program, a total of 31 institutions received support for training in 16 disciplines. The disciplines in which the largest numbers of awards were made are neurology (9 awards), neurophysiology (7 awards), and otolaryngology (6 awards). When these two-year awards terminate on June 30, 1978, the Institute will have honored all of its obligations to grantees supported through this program.

Postdoctoral Research Fellowship Program

This program, in which awards were made under the Weinberger authority, is in a phase-out status. Fourteen continuation awards were made this year and one additional year of recommended support to five of these awardees will be made next year. All of our commitments will then be honored.

Institutional and Individual National Research Service Award Programs

For most of the year these programs were in limbo because their legislative authority authorized an appropriation for only fiscal year 1975. On October 24, however, the NIH announced a continuation of these programs and by May, the required legislation (P.L. 94-278) was enacted and new dates for the receipt of applications were announced.

In the Institutional National Research Service Award Program, we had twelve active programs -- five in developmental neurology, five in sensory physiology and biophysics, and two in neurovirology -- which received their second year of recommended support. These programs had all received their initial year of support in FY '75. No awards were made in neuroimmunology or for the support of minority programs in the neurosciences. Forty-five new applications which were submitted to meet the January 2 deadline for the receipt of applications were assigned to the Neurology Institute. It is impossible to predict what action might be taken on these applications since many of them have not yet received their technical merit review. If any awards are made, it will reduce the number of NRSA's that are made to individuals.

During the year, the Institute was approved for making Institutional NRSA's in two new areas -- neurobiology and neuropathology/otopathology. This increased the number of training areas to seven and the 45 applications assigned to the Institute were distributed as follows:

Developmental Neurology	3
Minority Programs	3
Neurobiology	12
Neuropathology	16
Neurovirology	3
Sensory Physiology and Biophysics	8

Applications for Individual National Research Service Awards were evaluated at the November Council meeting. Of 101 applications for Individual Awards, 91 were recommended for approval and 10 were recommended for disapproval. Of the 91 approved applications, 15 were funded, 27 were carried forward to compete in June, 46 were administratively withdrawn, and three were withdrawn upon request by the applicant.

Following the June meeting of the Council, additional Individual National Research Service Awards will be made. The successful applicants will be selected from among the 27 applications being carried forward from the November Council and 305 new applications currently in the review process.

There were no changes in the Individual NRSA program during this year. However, when announcements of the continuation of this program are again distributed, we plan to add neuroepidemiology as a new training area.

Teacher-Investigator Program

At the beginning of the year, we had 18 Teacher-Investigator awardees, four of whom had no additional years of recommended support. At the March Council meeting, of 15 applications reviewed, 11 were recommended for approval and four were recommended for disapproval. Of the 11 approved applications, the Council recommended that six be funded making a total of 20 continuation and new Teacher-Investigator awards for FY '76.

Research Career Development Award Program

Research Career Development Award applications were reviewed at both the November and March Council meetings and 22 new applications were funded. During the fiscal year, 78 new and continuation awards had been made.

MANPOWER EVALUATION STUDIES

In the fall of 1972, the Institute awarded contracts to study manpower needs in speech pathology and audiology, otolaryngology, neurology, and neurological surgery. About a year later, a fifth contract was awarded to assess manpower needs in the basic sciences related to neurology and the communicative areas.

The manpower report in speech pathology and audiology was received a year ago. However, the reports for otolaryngology, neurology, and neurological surgery were received only this year. The manpower report in the neurological and communicative basic sciences has not yet been completed.

The Institute plans to have the Government Printing Office publish the four manpower reports in two volumes, one for the neurological reports and one for the communicative reports. A significant amount of time has been required to prepare the manuscripts for publication and it is anticipated that the reports in the communicative sciences will be ready for publication before the end of the fiscal year. An appreciable amount of additional work will be required before the neurology reports are in manuscript form.

ANNUAL REPORT
July 1, 1975 through June 30, 1976
Contracts Management Branch
Extramural Activities Program
National Institute of Neurological and Communicative Disorders and Stroke
National Institutes of Health

During the fiscal year the Contracts Management Branch (CMB) was established as an organizational entity at the Branch level. This action was in keeping with the proposed Institute reorganization plan which was approved during this reporting year.

The Contracts Management Branch consists of the Contracting Officer who is Chief of the Branch, three Contract Specialists and two supporting staff.

The CMB is charged with the administration of some 128 research contracts and Interagency Agreements totaling \$11.569 million. In addition there are fifty-eight (58) completed contracts in various stages of being closed out. The CMB expects to award approximately 15 new contracts this FY. Added to this workload is sixty-nine (69) renewals of existing contracts and over 80 actions modifying contracts which do not involve additional funds.

The Branch continues to make good progress in reducing the backlog of completed contracts that are not closed. During the reporting year authority was granted by HEW to the individual Contracting Officers to conduct fiscal audit on all completed contracts \$100,000 or less. Previously the amount was limited to \$50,000. Sixteen (16) completed contracts were administratively closed during the reporting year.

By far, the biggest single category of work in terms of labor-hours spent is caused by administration of the Privacy Act of 1974. In addition to the time spent in modifying the affected contracts by July 1, 1976, there is currently one contractor who steadfastly refuses to accept the fact that the Privacy Act applies to contracts with that institution. The matter is currently under review by the Institute Privacy Act Coordinator.

ANNUAL REPORT
July 1, 1975 through June 30, 1976
Extramural Activities
Grants Management Branch
National Institute of Neurological and Communicative Disorders and Stroke

The Grants Management Office has been reorganized to service the Extramural Activities by program area. Each area is serviced by a grants management specialist. All budget, policy and financial questions pertaining to a program area are referred to the assigned grants management specialist.

The new National Research Service Award budgets have required greater review prior to submission to their scientific review. Grantees have improperly interpreted the level of funds to be requested for Postdoctoral Stipends. This has resulted in a higher level of communication with the grantees to clarify their requests.

In the Program Project Grant area the Grants Management Office has been experiencing more involvement with the Program Staff in developing the approved budgets, by program area. The revised budget approvals and deletions furnished to the program directors reflect each approved program area, enabling them to better plan current and future budgets.

It has been requested of our office by the Scientific Evaluation Branch, to have a Specialist review an application prior to a site visit for relevant information, and at times attend the Project Site visit on the more involved Program Projects.

In view of the above and the additional involvement, our work load has increased to the level where the need is becoming greater for another grants management specialist.

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ANNUAL REPORT

July 1, 1975 through June 30, 1976

Scientific Evaluation Branch

National Institute of Neurological and Communicative Disorders and Stroke

The Scientific Evaluation Branch (SEB) was established in 1975 and assigned responsibility for the technical merit and scientific review of Contracts, Program Projects, Clinical Research Centers, Outpatient Research Centers, Institutional Fellowships and Teacher-Investigator Awards. The Communicative Disorders Review Committee, Neurological Program Project Committee A, Neurological Program Project Committee B, and Special Review Committee are components of the SEB and provide the initial technical merit and scientific review of the proposals for the Institute. Summary statements are prepared by the SEB for the Institute staff, Contract Review Board and/or the Institute National Advisory Council.

The SEB has established liaison with the Intramural Program of the Institute, the Communicative Disorders Program, Fundamental Neuroscience Program, Neurological Disorders Program, and the Stroke and Trauma Program to facilitate the technical merit and scientific review of contracts, grants and fellowships. The SEB maintains a continuing liaison with leaders of the communicative and neurological disorders scientific community for the purpose of identifying the most qualified persons to serve on SEB panels and committees.

ANNUAL REPORT

July 1, 1975 through June 30, 1976

Extramural Activities Program

Data Analysis and Reports

National Institute of Neurological and Communicative
Disorders and Stroke

The Office of Data Analysis and Reports, with its computerized information retrieval system in parallel with a manual system, is fully operational for research and training grants, fellowship awards and institutional trainee appointments. Since contracts were newly assigned to NINCDS-EA we have not yet acquired the contracts data in our files. The operational status of all previous data systems has been maintained, despite the great surge of reclassification workload and the added retrieval activity occurring as a result of shifting managerial responsibilities associated with the NINCDS reorganization. Much of the new activity and workload is permanent, and was anticipated. This caused us to train one more person in the reclassification processes and upgrade that position. To date the major shifts of grants to new program areas has been managed without loss of data comparability, i.e., numbers of grants and dollars awarded, by disorder grouping or scientific discipline. Comparability usually is quite essential for confidence in data accuracy when depending on computer retrieval information.

This group prepares data books, fiscal year-end summary books, special reports, and verbally presented fiscal trend reports to Council, as well as a frequently updated listing of all targeted research grants. Most of these types of DAR output have been improved somewhat, but some changes are significant and warrant further elaboration because of the increased management utility they provide and because of the workload implications. For example, the data books were improved in format and will be published prior to Council rather than after it. Our group will conduct a thorough study of the DRG contracts data for NINCDS, and then will set up a storage and retrieval system, compatible with DRG's if possible. These data will be added to the data book when the edit is done and the formats have been developed and programmed.

Another major data extraction, storage, edit, and retrieval task is underway and will be finished this fiscal year, but will not be incorporated in any regularly produced DAR documents. This task deals with program projects and clinical centers and all of their included subprojects along with the associated classification of these often multifaceted grants in order to assure a more efficient transition of responsibility from technical merit review to program area and vice versa. This task is addressed to this need. Unfortunately the DRG data base is not accurate enough in reflecting reality because of its dependence on the applications rather than the site visit reports or summary statements. The task thus will be more onerous in proportion to the number of grants involved. These two new data management undertakings probably will mean that we will need our part-time STRIDE

employee on a full-time basis when she finishes that study program.

No unusual delays or data management problems are anticipated, assuming administrative support in improving the computer printing and courier services, and assuming that revision of the NINCDS classification system does not impose a greatly increased workload.

Table of Organization

Intramural Research Program

National Institute of Neurological and Communicative Disorders and Stroke
(Personnel on hand May 1976)

OFFICE OF THE DIRECTOR

Director, Intramural Research Program - Thomas N. Chase, M.D.
Laboratory Director, IRP - Richard L. Irwin, Ph.D.
Clinical Director, NINCDS - Donald B. Calne, M.D.
Medical Officer - Jacob A. Brody, M.D.
Medical Officer - Choh-Luh Li, M.D.
Administrative Officer - Glenn E. Hammond
Administrative Assistant - J. Loring Jenkins
Administrative Assistant - John H. Jones
Administrative Technician - Doris R. Perry
Secretary (Stenographer) - Margaret Henry
Secretary (Stenographer) - Vernita Bergmeyer
Travel Assistant - Ida M. Chernikoff
Biological Laboratory Technician (Animal) - George R. Duvall
Biological Laboratory Technician (Animal) - Adrian P. Loftis
Biological Laboratory Technician - Alexander Wheaton
Procurement Assistant - Patricia D. Williams
Biologist - Minnie Toure
Secretary (Stenographer) - Corinne C. Gillis
Administrative Technician - Joan K. Russo
Program Analyst - Andrea C. Gielen
Secretary (Stenographer) - Doris Selkowitz
Secretary (Typing) - Lillian L. Wease
Biological Laboratory Technician (Micro) - Charles E. Sartor
Budget Clerk (Typing) - Carolyn L. Peel
Clerk (DMT) - Pamela Barnicoat
Clerk-Stenographer - Joan E. Kraft
Clerk (Typing) - Elizabeth Bloom
Clerk (Typing) - Dorothy Mudrick
Clerk (Typing) - Beth A. Garman
Laboratory Worker - John R. Bowers
Laboratory Worker - Kenneth Oglesbee
Biological Aid (Micro) - Asela Russell

Section on Technical Development

Computer Systems Analyst - William H. Sheriff, Jr.
Staff Fellow - Bruce Smith, Ph.D.
Staff Fellow - Susan Hauser, Ph.D.
Engineering Technician - Michael J. Walsh

NINCDS Guam Research Center

Medical Officer - Paul M. Hoffman, M.D.
Health Technician - Jose M. Torres
Health Technician - Manuel T. Cruz
Health Technician - Francisco Leon-Guerrero
Nurse - Marjorie Gillespie
Biological Laboratory Technician (Micro) - Luiz T. Munoz
Secretary (Typing) - Mary E. Hernandez

MEDICAL NEUROLOGY BRANCH

Office of the Chief

Chief, Medical Neurology Branch - W. King Engel, M.D.
Surgeon - Barry W. Festoff, M.D.
Biological Laboratory Technician (Micro) - Guy G. Cunningham
Physiologist - E. Carolyn Derrer
Biologist - Mary A. Oberc
Microbiologist - Priscilla Chauvin
Surgeon - Jay D. Cook, M.D.
Surgeon - John L. Trotter, M.D.
Surgeon - Sidney C. Bean, M.D.
Surgeon - Benjamin Brooks, M.D.
Surgeon - Roger A. Brumback, M.D.
Surgeon - John W. Gittinger, M.D.
Surgeon - Roger W. Kula, M.D.
Surgeon - Robert Shebert, M.D.
Visiting Associate - Tulio Bertorini, M.D.
Visiting Associate - Neelupalli Reddy, M.D.
Visiting Fellow - Albert Dubrovsky, M.D.
Secretary - Lucy L. Riley
Biologist - Helen J. Osborne
Biological Laboratory Technician - Jane V. Lawrence
Biological Laboratory Technician - Gregory C. Zirzow
Biologist - William Creegan
Biological Laboratory Technician - Gregory Hubbard
Biological Laboratory Technician - Joseph Sciabarrasi
Secretary (DMT) - Gertrude Wright
Biological Laboratory Technician - Samuel T. Carroll
Clerk (DMT) - Anne K. Lawrence
Histopathology Technician - Thelma Fletcher
Biological Laboratory Technician - James Chorbajian
Biological Aid (Micro) - Brian K. Zell
Clerk-Typist - Beverly Skelton
Clerk-Typist - Alberta L. Huff
Biological Aid - Margaret Vaughan
Laboratory Worker - Matthew P. Meadows
Biological Aid - Janet M. Harner
Biological Aid - Henry T. Fones

Biological Aid - Robert Vanderbilt

Clinical Applied Pharmacology Section

Biologist - Katherine Oliver
Secretary (DMT) - Emma P. Dick

SURGICAL NEUROLOGY BRANCH

Office of the Chief

Acting Chief, Surgical Neurology Branch - Ayub K. Ommaya, F.R.C.S.
Surgeon - Ronald Uscinski, M.D.
Surgeon - James H. Wood, M.D.
Staff Fellow - Earl C. Mills, M.D.
Staff Fellow - Paul C. Williams, M.D.
Clerk (DMT) - Helen M. Andregg
Secretary (Steno) - Olga G. Williams
Clerk-Typist - Raymond F. Weaver

Applied Research in Surgical Neurology Section

Biological Laboratory Technician (Biochem) - M. Arthur Banks
Biological Laboratory Technician (Animal) - Calvin S. Hawkins
Biological Laboratory Technician (Animal) - Clifford A. Seay
Biological Aid - Stephen O'Donnell

Neuroradiology Section

Research Medical Officer - Giovanni Di Chiro, M.D.
Sr. Staff Fellow - Mary K. Hammock, M.D.
Sr. Staff Fellow - Rodney A. Brooks, M.D.
Clerk-Typist - M. Jacqueline Pattison
Clerk-Typist - Eileen Yellin
Biological Aid - Carlton Lampkins

CLINICAL NEUROSCIENCES BRANCH

Office of the Chief

Chief, Clinical Neurosciences Branch - Cosimo Ajmone Marsan, M.D.
Clerk-Stenographer - Myrtle Sullivan
Clerk (DMT) - Tina McDaniels

Clinical Neurophysiology Section

Surgeon - Donald W. King, M.D.
Surgeon - Boris A. Vern, M.D.
Surgeon - James B. Macon, M.D.

EEG Technician - Joan Trettau
Biologist - Stuart Walbridge
EEG Technician - Martha H. Fair

Functional Neurosurgery Section

Associate Chief, Clinical Neurosciences Branch - John M. Van Buren, M.D.
Research Psychologist - Paul Fedio, Jr., Ph.D.
Biologist - Rosemary C. Borke
Histopathology Technician - Martha L. Johnson
Psychologist - Ghislaine R. Frederick
Histopathology Technician - Vivian A. Betton
Surgeon - David M. Bear, M.D.
Clinical Psychologist - John Oubre
Clerk-Stenographer - Elizabeth Bliss

DEVELOPMENTAL AND METABOLIC NEUROLOGY BRANCH

Office of the Chief

Chief, Developmental & Metabolic Neurology - Roscoe O. Brady, M.D.
Secretary (Stenographer) - Barbara W. Critzer
Clerk (DMT) - Lois A. Brown
Clerk-Typist - Shelia Y. Burris
Visiting Fellow - Ricardo Duffard, Ph.D.
Visiting Fellow - Rosemary McIntyre, Ph.D.

Enzymology and Genetics Section

Research Chemist - Richard Qualres, Ph.D.
Research Chemist - Peter H. Fishman, Ph.D.
Research Chemist - Peter G. Pentchev, Ph.D.
Chemist - Roy M. Bradley
Chemist - Jane M. Quirk
Biological Laboratory Technician - George E. Mook
Staff Fellow - John W. Kusiak, Ph.D.
Visiting Fellow - Laurence McIntyre, Ph.D.

Neurochemical Methodology Section

Research Chemist - Andrew E. Gal, Ph.D.
Biological Laboratory Technician - Frank J. Fash

Clinical Investigation and Therapeutics Section

Assoc. Chief, Developmental & Metabolic Neuro. - Anatole Dekaban, M.D.
Research Chemist - George Constantopoulos, Ph.D.
Medical Technician - Jan K. Steusing
Surgeon - Michael P. Whyte, M.D.
Visiting Associate - Norio Sakuragawa, M.D.

LABORATORY OF NEUROPATHOLOGY AND NEUROANATOMICAL SCIENCES

Office of the Chief

Chief, Neuropathology & Neuroanatomical Sciences - Igor Klatzo, M.D.
Biological Laboratory Technician (Animal) - Willie Perkins
Secretary (DMT) - Virginia Masterson
Biologist - Robert S. Graham
Biological Laboratory Technician (Animal) - Albert V. Cantu
Biological Laboratory Technician (Animal) - Melvin H. Carroll
Clerk-Stenographer - Patricia A. Bustin
Clerk-Typist - S. Joan Eskin
Visiting Fellow - Tsukasa Fujimoto, M.D.
Visiting Fellow - Dejan V. Micic, Ph.D.

Functional Neuroanatomy Section

Medical Officer - Thomas S. Reese, M.D.
Biological Laboratory Technician - Frank D. Nolan
Biological Laboratory Technician - Linda J. Perry
Visiting Fellow - Rosemary P. Rees, Ph.D.

Neurocytology Section

Research Physiologist - Milton Brightman, Ph.D.
Staff Fellow - Richard Broadwell, Ph.D.
Histopathology Technician - Gertrude Goping
Biological Laboratory Technician - Barbara F. Reese
Clerk-Typist - Katy Davis
Visiting Fellow - Masayasu Sato, M.D.

Experimental Neuropathology Section

Medical Officer - Jan Cammermeyer, M.D.
Biologist - Iris Fenton
Editorial Assistant - Jane T. Phelps
Biological Aid - Sophia Grabinski

Cerebrovascular Pathology Section

Biological Laboratory Technician - Joseph T. Walker

Cellular Neuropathology Section

Assoc. Chief, LNNS - Henry DeF. Webster, M.D.
Biological Laboratory Technician - Maureen O'Connell
Histopathology Technician - Kathryn Winchell
Biological Aid - Susan Larrick
Staff Fellow - Michael J. Cullen, Ph.D.
Biological Aid - Nancy A. Newkirk
Visiting Fellow - Takeshi Tabira, M.D.

Cellular Neurochemistry Section

Research Biologist - Janet Passonneau, Ph.D.
Sr. Staff Fellow - Wesley D. Lust, Ph.D.
Biologist - Sandra K. Crites
Biological Aid - Ann V. Deaton
Biological Aid - Karen Wayns
Visiting Fellow - Masashi Kobayashi, M.D.
Visiting Associate - Maria V. Gentina

Neurocytobiology Section

Medical Officer - Maria Spatz, M.D.
Research Biologist - Margaret Murray, Ph.D.
Biological Laboratory Technician - Madora E. Swink
Biologist - Joliet Y. Bemby

LABORATORY OF NEUROPHYSIOLOGY

Office of the Chief

Chief, Laboratory of Neurophysiology - Michelango Fuortes, M.D.
Electronics Technician - Anthony F. Bak
Biological Laboratory Technician - William L. Beane
Clerk (DMT) - Gloria K. Hall
Clerk-Typist - Daveen Y. Strahle
Visiting Scientist - Luigi A. Cervetto, M.D.
Visiting Fellow - Jiri Pochobradsky, Ph.D.

General Physiology Section

Chemist - Patricia Grimes
Visiting Scientist - Franco Conti, Ph.D.

Sensory Physiology Section

Medical Officer - Thomas G. Smith, M.D.

Cell Biology Section

Research Biologist - Arnaldo Lasansky, M.D.
Biological Laboratory Technician - Julia M. Lohr
Staff Fellow - Richard Normann, Ph.D.
Visiting Fellow - Menachem Hanani, Ph.D.

Neuronal Interactions Section

Head, Neuronal Interactions Section - Henry G. Wagner, M.D.
Sr. Staff Fellow - Ferenc I. Karosi, Ph.D.
Sr. Staff Fellow - Helga E. Stanbury, Ph.D.

LABORATORY OF BIOPHYSICS

Office of the Chief

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Visiting Associate - Tadashi Akaike, M.D.
Staff Fellow - Eliahu Heldman, Ph.D.
Clerk (Typing) - Hazel W. Fermino
Visiting Fellow - Bernard Pailthorpe, Ph.D.

Neural Membranes Section

Research Biophysicist - Kenneth S. Cole, Ph.D.
Research Physiologist - Jay B. Wells, Ph.D.
Visiting Fellow - Robert J. French, Ph.D.

Neural Systems Section

Surgeon - Daniel L. Alkon, M.D.
Visiting Fellow - Yoram Grossman, Ph.D.

Molecular Biophysics Section

Acting Head, Molecular Biophysics Section - Gerald Ehrenstein, Ph.D.
Supervisory Research Physiologist - Robert E. Taylor, Ph.D.
Research Physicist - Richard Fitzhugh, Ph.D.
Research Physiologist - Daniel L. Gilbert, Ph.D.
Electrical Engineer - Leonard Binstock
Research Physicist - Harold Lecar, Ph.D.
Mathematician - John Shaw
Electronic Technician - Herbert A. Walters
Secretary (Stenographer) - Maxine Schaefer
Biological Aid - Sandra I. Dixon
Biological Laboratory Aid - Samuel C. Hurwitz
Biological Aid - Norman Magid
Visiting Fellow - Lida Firouzi, Ph.D.

LABORATORY OF NEUROCHEMISTRY

Office of the Chief

Acting Chief, Laboratory of Neurochemistry - R. Wayne Albers, Ph.D.
Surgeon - Lawrence Rodichok, M.D.
Clerk (Typing) - Sheri L. Carson
Clerk-Stenographer - Sylvia Levinson
Clerk-Typist - Jacqueline Childs
Biological Aid - Linda A. Garrett

Amino Acid Chemistry Section

Chemist - Oscar M. Young
Staff Fellow - Stephen Goldman, Ph.D.

Enzyme Chemistry Section

Chemist - George J. Koval
Visiting Associate - Dou H. Jean, Ph.D.
Physical Science Technician - Eunice L. Summers

Physiology Metabolism Section

Research Chemist - Eberhard G. Trams, Ph.D.
Chemist - Carl J. Lauter
Staff Fellow - Dorri Rosenblatt, Ph.D.

Neuronal Development and Regeneration Section

Biological Laboratory Technician - Janina D. Ziemnowicz
Surgeon - Andrew Zalewski, M.D.
Sr. HSO - George F. Creswell

LABORATORY OF MOLECULAR BIOLOGY

Office of the Chief

Chief, Laboratory of Molecular Biology - Ernst Freese, Ph.D.
Visiting Associate - Chang-Chih Tai, Ph.D.
Staff Fellow - Peter Cooney, Ph.D.
Staff Fellow - Kennerh E. Rich, Ph.D.
Staff Fellow - Barbara C. Levin, Ph.D.
Secretary (Stenographer) - Geraldine Bitango
Clerk-Typist - Donna L. Hanyo
Clerk-Typist - Mary L. Keplinger
Visiting Fellow - Tadako Iijima, Ph.D.
Visiting Fellow - Patricia Whiteman, Ph.D.
Visiting Fellow - Ayub K. Yousufi, Ph.D.

Developmental Biology Section

Research Biologist - Elisabeth G.M. Freese, Ph.D.
Chemist - Enid Galliers
Chemist - Cheryl Marks
Visiting Associate - Robert A. Pearce, Ph.D.
Sr. Staff Fellow - Richard C. Henneberry, Ph.D.
Biological Aid - Abraham Rosner
Visiting Fellow - E. East Atikkan, Ph.D.
Visiting Fellow - Kazuhito Watabe, Ph.D.
Visiting Fellow - Yasutaro Fujita, Ph.D.

Molecular Virology Section

Research Chemist - Robert A. Lazzarini, Ph.D.
Chemist - Leslye E. Johnson
Staff Fellow - Jack Keene, Ph.D.
Staff Fellow - Marie L. Dirksen, Ph.D.
Biological Aid - Stephen I. Adler
Biological Aid - Shelia Carroll
Biological Aid - David R. Hardy
Laboratory Worker - Margaret L. Shea
Visiting Fellow - Toshiro Adachi, Ph.D.

LABORATORY OF PERINATAL PHYSIOLOGY

Office of the Chief

Chief, Laboratory of Perinatal Physiology - Ronald E. Myers, M.D.
Research Psychologist - Shun-ichi Yamaguchi, Ph.D.
Biological Laboratory Technician - Esteban Monell-Torrens
Biological Laboratory Technician - William Rodriguez
Surgeon - William F. Blank, M.D.
Visiting Associate - Michio Yamaguchi, M.D.
Chemist - Carmen L. Freixas
Secretary (Stenographer) - Elizabeth Demshock

LABORATORY OF NEURAL CONTROL

Office of the Chief

Acting Chief, Laboratory of Neural Control - Robert E. Burke, M.D.
Biological Engineer - Edward M. Schmidt
Research Physiologist - William B. Marks, Ph.D.
Engineering Technician - George M. Dold
Electronics Engineer - Martin J. Bak
Physiologist - Joan S. McIntosh
Surgeon - Gerald E. Loeb, M.D.
Staff Fellow - John S. Thomas, Ph.D.
Clerk (DMT) - Nancy Cavendish
Laboratory Worker - Danessa Drumgold
Visiting Fellow - Kendro Kanda, M.D.
Visiting Fellow - Bruce Walmsley, Ph.D.

LABORATORY OF NEURO-OTOLARYNGOLOGY

Office of the Chief

Chief, Laboratory of Neuro-Otolaryngology - Jorgen Fex, M.D.
Biologist - Gabrielle Neises
Electronics Engineer - William Livingston
Chemist - Anne J. Mustafa
Sr. Staff Fellow - Robert L. Gulley, Ph.D.
Sr. Staff Fellow - Dennis Drescher, Ph.D.
Staff Fellow - Joe C. Adams, Ph.D.
Staff Fellow - Robert Wenthold, Ph.D.
Secretary (Stenographer) - Elsie Walter
Histopathology Technician - Barbara B. Judy
Histopathology Technician - Marianne Parakkal
Biologist - Susanna W. Jones
Clerk-Typist - Doreen Dalessandro

LABORATORY OF EXPERIMENTAL NEUROLOGY

Office of the Chief

Chief, Laboratory of Experimental Neurology - William Caveness, M.D.
Chemist - Samuel Speciale, Ph.D.
Visiting Associate - Shinich Wakisaka, M.D.
Chemist - Raymond R. O'Neill
Biologist - Barbara Malamut
Secretary (Typing) - Marguerite Smiley
Biological Aid - Julia Garfinkle
Visiting Scientist - Motohiro Kato, M.D.
Visiting Fellow - Chinich Hosokawa, M.D.

NEUROIMMUNOLOGY BRANCH

Office of the Chief

Chief, Neuroimmunology Branch - Dale E. McFarlin, M.D.
Chemist - Elizabeth Mingioli
Medical Technologist - Carolyn A. Pedone
Sr. Surgeon - Henry McFarland, M.D.
Sr. Staff Fellow - Hillel S. Panitch, M.D.
Staff Fellow - Leslie B. Barnett, M.D.
Staff Fellow - Eugene D. Johnson, Ph.D.
Staff Fellow - Joseph F. Poduslo, Ph.D.
Secretary (Stenographer) - Shirley A. Burdette
Chemist - William Tucker
Biological Aid - Andrew F. Carlson
Clerk-Typist - Karen L. Beard
Biological Aid - Jody M. Wildy
Laboratory Worker - Donna S. Walters

LABORATORY OF CENTRAL NERVOUS SYSTEM STUDIES

Office of the Chief

Chief, Lab. of Central Nervous System Studies - Carleton Gajdusek, M.D.
Supervisory Research Microbiologist - Clarence J. Gibbs, Ph.D.
Medical Officer - David M. Asher, M.D.
Biological Laboratory Technician - Michael P. Sulima
Biological Laboratory Technician - John L. Priester
Biological Laboratory Technician - Alfred E. Bacote
Microbiologist - Nancy P. Luber
Social Science Analyst - Judith B. Meyer
Anthropologist - Laura A. Kreiss
Microbiologist - Monica Ann Lewis
Director - Paul W. Brown, M.D.
Senior Surgeon - Lon R. White, M.D.
Surgeon - Richard Yanagihara, M.D.
Veterinarian - Herbert L. Amyx, D.V.M.
Sr. Staff Fellow - George J. Nemo, Ph.D.
Staff Fellow - Ralph M. Garruto, Ph.D.
Staff Fellow - Robert G. Rohwer, Ph.D.
Anthropologist - Richard Benfante
Biological Laboratory Technician - Helena L. Gilbert
Secretary (Stenographer) - Marion F. Poms
Mathematician - Steven G. Ono
Secretary (DMT) - N. LaDonna Tavel
Translator - Jose Figirlioyong
Clerk (DMT) - Wendy W. Showers
Biological Laboratory Technician - Randolph Taylor
Biological Aid - Jay R. Gorham
Biological Aid - Ann C. Rudnick
Biological Aid - Sylvia Anderson
Biological Aid - Judith P. Chavis
Biological Aid - Michael N. Kent
Social Science Aid - Amy Levin
Health Technician - Ivan M'Baginatao
Animal Caretaker - Hubert O. Saville
Animal Caretaker - Ezra Shafer
Animal Caretaker - Harry Winpigler
Animal Caretaker - Leon J. Vance
Animal Caretaker - Eugene D. Webster
Biological Laboratory Technician - Holly T. Dublin
Biological Aid - Karin A. Grimes
Biological Laboratory Technician - Margaret A. Parry
Biological Laboratory Technician - Tanya K. Ross

LABORATORY OF NEUROPHARMACOLOGY

Office of the Chief

Sr. Staff Fellow - Judith R. Walters, Ph.D.
Chemist - Nancy Eng
Histopathology Technician - Raymond R. Vane
Sr. Surgeon - William Lightfoote, M.D.
Surgeon - Ronald Kartzinell, M.D.
Visiting Associate - Paul F. Teychenne, M.D.
Sr. Staff Fellow - Mark J. Perlow, M.D.
Staff Fellow - Ellen Silbergeld, Ph.D.
Staff Fellow - Leonard P. Miller, Ph.D.
Secretary (Typing) - Sandra M. Meadows
Biologist - Sandra DeSantis
Biological Laboratory Technician - Joan M. Lakoski
Biological Aid - Shawn W. Kennedy
Biological Aid - Mildred Nelson
Clerk-Typist - Regina Starr

INFECTIOUS DISEASES BRANCH

Office of the Chief

Chief, Infectious Diseases Branch - John L. Sever, M.D.
Secretary (Stenographer) - Ethel M. Ingram
Procurement Clerk - Florence Reid
Secretary (DMT) - Nelva L. Reckert
Clerk-Typist - Sharon K. Painter
Clerk (DMT) - Daryl Dawson

Experimental Pathology Section

Veterinarian - William T. London, D.V.M.
Biologist - Blanche Curfman
Biological Laboratory Technician - Robert L. Brown
Biological Laboratory Technician - Geneva M. Brown
Research Veterinarian - Amos E. Palmer, D.V.M.
Biological Laboratory Technician - Joseph Ricketts
Biological Aid - Wayne E. Nusbaum
Biological Aid - Sherry L. Thomas
Biological Aid - Dorene Conley
Animal Caretaker - Adley J. Atkinson
Laboratory Worker - Douglas Sykes
Laboratory Worker - Claude Belcher
Laboratory Worker - Delter Catron
Laboratory Worker - Carl Rollison
Clerk-Typist - Betty I. Boone

Immunochemistry and Clinical Investigations Section

Microbiologist - Anita Ley
Nurse - Dorothy Edmonds
Sr. Staff Fellow - Michael F. Murphy, Ph.D.
Biological Laboratory Technician - Paul P. Becher
Biological Laboratory Technician - Gene M. Brashears
Medical Technician - Jennifer Dorosz
Medical Technician - Ampar Strickland
Clerk - Frederick Brownholtz
Visiting Fellow - Morag Ferguson, Ph.D.

Neurovirology Section

Senior Scientist - David A. Fuccillo, Ph.D.
Research Microbiologist - Maneth Gravell
Microbiologist - Flora Moder
Microbiologist - Renee G. Traub
Nurse Specialist - Helen M. Krebs
Biologist - Rebecca Hamilton
Biological Laboratory Technician - Aurellia S. Krezlewicz
Biologist - Kenneth Rich
Biological Laboratory Technician - Frank J. West
Biologist - Sandra Fitzgerald
Microbiologist - Mary A. Krasny
Biological Laboratory Technician - Janet M. Mattson
Veterinarian - David L. Madden, D.V.M.
Microbiologist - Otto Gutenson
Biological Aid - Leonard Moore
Biological Aid - Mitchell Binder
Biological Aid - Kenneth A. Blank

Neurogenetics Section

Senior Surgeon - Roswell Eldredge, M.D.
Technical Research Assistant - Thelma Koerber
Clerk-Typist - Valerie Washington
Psychology Aid - Nancy Tang
Clerk-Typist - Adolphus L. Groom
Clerk-Typist - Shelia Riggans

Electron Microscopy Section

Visiting Scientist - Monique Dubois, Ph.D.
Histopathology Technician - Edna Worthington
Visiting Fellow - Marcos Rodriguez, Ph.D.

Annual Report of the Scientific Director
of the
National Institute of Neurological and
Communicative Disorders and Stroke
July 1, 1975 through June 30, 1976

The Intramural Research Program of the National Institute of Neurological and Communicative Disorders and Stroke conducts research related to the nervous system and its disorders in its Bethesda laboratories and clinics as well as in facilities at Fort Detrick, Maryland, at Woods Hole, Massachusetts, and at the Guam Research Center. Within this program, a broad array of basic and clinical techniques are applied to studies embracing nearly every aspect of the form and function of the nervous system.

The past year witnessed no major organizational changes, but rather marked the successful completion of a number of previously initiated modifications. Grouping of the Intramural Program's 17 laboratories and Branches into fundamental or clinically applied divisions, each headed by an appropriately designated line official, has functioned exceptionally well. The three recently established laboratories (Neuroimmunology, Neuropharmacology, and Neuro-otolaryngology) are now fully operational, as is the newly reorganized Clinical Neurosciences Branch. Sections of the Laboratory of Biophysics, transferred late last year to the Marine Biological Laboratories in Woods Hole, are beginning to function effectively. The Guam Research Center has been strengthened by a more focused research effort, bolstered by the addition of professional staff and new equipment.

Personnel and budgetary constraints continue to compel vigorous measures for optimizing internal resource distribution and utilization. These endeavors were most conspicuous in the area of personnel, which remained the most challenging problem faced by the Intramural Research Program. On May 1, 1976, 476 individuals worked in the Program, an increase of 53 over the preceding year's total. However, this increase largely reflects strenuous attempts to recruit ceiling-free personnel, since imposition of a partial hiring freeze decreased budgeted staff by 3%. This reduction, plus the award of 19 new positions, will enable the Intramural Program to be within its new ceiling for budgeted personnel by the close of the current fiscal year. Nevertheless, hiring restrictions preferentially reduced the number of younger, non-tenured physicians and scientists. Partial compensation derived from a dramatic expansion in ceiling-free personnel: visiting fellows, guest workers and stay-in-school employees increased 69%. In addition, the Intramural Program took advantage of the Inter-governmental Personnel Act by recruiting 7 talented scientists from colleges and universities to work temporarily at the Bethesda campus.

Administrative actions to improve the pattern of professional staffing also operated to the Program's advantage. The drift towards a largely tenured professional staff was reversed with the departure of 9% of the tenured doctoral level personnel and attempts to discourage the conversion of younger scientists to tenured status. But the hiring embargo decreased non-tenured doctoral level personnel by nearly the same amount. On the other hand, the Intramural Program succeeded in expanding the number of ceiling-free physicians and scientists by

73%, thus enabling a 6% increase in the total doctoral staff. Technical and administrative support staffing grew by 26% during the year with a 7% increase in budgeted and a 34% increase in non-ceiling support employees. Accordingly, the ratio of total professional to total support personnel, which had gradually declined in recent years to inefficiently low levels, began to improve.

On-campus research facilities expanded by 1100 sq. ft. during the year through transfer of Building 36 modules vacated by the Collaborative and Field Research Program. However, failure to recover loaned space in Building 36 continues to delay implementation of an extensive and critically needed space reallocation plan. Furthermore, limitations on the availability of research beds in the Clinical Center still pose serious difficulties to the Intramural Program. It is hoped that rapid implementation of a Bed Banking plan to facilitate inter-institute sharing of clinical facilities, and the eventual availability of the Ambulatory Care Research Facility will alleviate this impediment to clinical research. Renovations of Building 376 at Fort Detrick are nearing completion, and transfer of viral research operations to this facility are expected within the coming year.

The annual Intramural allocation for "other objects" was \$5.1 million, a decrease of \$.6 million from the preceding fiscal year. Although adequate in amount, delay in receiving the full apportionment until the third quarter caused stringencies and operating inefficiencies for several laboratories. Research contracts sponsored by intramural scientists received an allotment of \$2.7 million, down \$0.6 million from the preceding year.

During the year, 227 research projects were conducted, 40 new projects initiated, 20 completed, and 21 terminated. Collaborative efforts were carried out with 34 other federal agencies and 146 non-governmental institutions. Based on the judgment of scientists reporting individual research projects, 40% of the Intramural Program's other objects funds went to fundamental research, while 60% was devoted to clinically applied studies. Neurophysiology, neurochemistry and neuroanatomy received the largest budgetary allocations in the basic neurosciences, while applied studies of infectious (especially virologic), neuromuscular, vascular, and demyelinating disorders were accorded the largest support in the clinical neurosciences. On the basis of research bed utilization in the NIH Clinical Center, studies of neuromuscular, convulsive, and extrapyramidal disorders remained most active.

By all available criteria, Intramural scientists enjoyed an exceptionally productive year. During 1975, 213 research reports appeared in 108 different scientific journals or books. Physiology, pathology, chemistry and virology/immunology were represented by the greatest number of publications. Neurology, Advances in Neurology, Brain Research, Journal of Infectious Diseases, Biochimica et Biophysica Acta, Journal of General Physiology, Lancet, and Experimental Neurology, in descending order, published the greatest number of Intramural scientist authored articles. Seven Intramural researchers contributed chapters to the Institute's 25th Anniversary Volumes, "The Nervous System." In addition, numerous Intramural scientists were invited to present lectures or seminars before learned society and university audiences in this country and abroad. Others were elected to the editorial boards of prestigious

journals and to the scientific advisory boards of various health voluntary and professional organizations. Among those accorded public recognition for outstanding scientific accomplishment were eight Intramural staff members given the DHEW Distinguished Service Award or the PHS Meritorious Service Award. Finally, rigorous triannual evaluations of four Laboratories by the Institute's Board of Scientific Counselors augmented by Ad Hoc specialists yielded highly laudatory comments as well as priority scores averaging substantially superior to those currently required by the Institute for funding of research grants.

During the past year, new knowledge development by Intramural scientists in both basic and clinical neurosciences continued at an astonishing pace. These findings are adequately detailed within the individual laboratory summaries and accompanying project reports. Only a few selected examples, which seem of special relevance to future progress, are adumbrated here.

The Laboratory of Molecular Biology has been investigating the cellular effects of lipophilic acids, which include preservatives, antiseptics, and compounds known to cause brain damage. These substances inhibit the growth of mammalian cells by a general effect whose potency increases with the lipophilicity of the compound. One short chained lipophilic acid, butyrate, has been found to produce reversible morphologic changes and induce alkaline phosphatase and sialyltransferase (the latter needed for synthesis of the sphingolipid GM₃) in HeLa cells. Another lipophilic acid, hexachlorophene, was shown to inhibit bacteria by destroying the proton gradient across the cell membrane and thereby starving them for substrate. The compound binds so tenaciously to cells that it can only be washed off with highly lipophilic polymers, such as serum albumin, suggesting that treatment for persons exposed to highly lipophilic toxins might include repeated replacement of their serum albumin or washing their blood with activated charcoal.

It is well known that members of the myxo-, paramyxo-, and rhabdovirus family can enter the central nervous system normally or as a complication of a systemic viral infection, and thus cause meningitis or encephalitis. Some of the infections or their consequences appear to persist for years. These RNA viruses are uniquely capable of producing defective particles which interfere with multiplication of normal virus. While this autointerference may be essential for the nonlethal outcome of a virus infection, it may also be responsible for long-term persistence. At least two phenomena now appear to contribute to the interference of normal virus development by the defective virus: first, different defective viruses seem to contain the same 3' and 5' end of the RNA sequence; using one or both these ends, they bind to the viral replicase and thereby reduce the synthesis of normal virus RNA. Second, mRNA needed for viral protein synthesis seems to be transcribed only from the normal virus RNA; thus the total number of viruses (infective and noninfective) into which the RNA molecules can mature is reduced by interference with viral protein synthesis. Viral persistence could not be related to the presence of virus derived DNA in the cellular chromosome, but rather seemed to depend on the continued presence of viral RNA itself.

The Laboratory of Neural Control has focused research efforts on mammalian central nervous system mechanisms involved in the control of movement. De-

velopment of novel methods for obtaining recordings from experimental animals which are awake and behaving with minimal restraint is an important aspect of this research. Although the development of equipment and techniques for making connections with the nervous system is rarely an end in itself, but rather occurs in relation to a specific research problem, such efforts have proven useful to investigators throughout the country.

Recent observations of impulse activity in spinal neurons after cutaneous nerve stimulation indicate that recruitment patterns of alpha motoneurons within a given nucleus are controlled largely by factors related to "synaptic organization" rather than to properties intrinsic to the cells themselves. The Laboratory's demonstration that large changes in recruitment patterns can occur in response to a single input system suggests that motoneuron "excitability" and recruitment order are properties of the way in which synaptic input is organized to particular neurons in the motor pool as well as to the interaction of such organizational factors with intrinsic motoneuron properties such as input resistance, time constant, and absolute voltage threshold.

An experimental design has been developed to answer the question of cell movement specificity. Monkeys trained to alter cortical cell firing rates to get rewards manage by producing various movements. Cortical neuron use not correlated to any movement is not rewarded. In this situation monkeys, working with a particular cell over a long time period, "learn" to redefine their movements to include only those appropriate to the neurons being studied.

The Laboratory of Neurochemistry has continued diversified efforts to explain neural mechanisms in terms of molecular events. New data has been obtained on physical parameters influencing the equilibrium between the two conformations of $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ that differ in the orientation of the K^+ ionophor. Measurements of the phosphorylation rate of this enzyme suggest the existence of a hitherto unidentified intermediate in the hydrolysis of ATP. Studies in mice varying in susceptibility to audiogenic seizures indicate that seizure-prone animals exhibit relatively low calcium-stimulated ATPase activity. From observations on the properties of this enzyme as a function of age, it was inferred that several different isoenzymes may appear at different stages of brain maturation. Investigation of factors permitting the active transport of sodium and potassium to operate with increased efficiency at low temperatures has implicated changes in the lipid composition of plasma membranes. Measurements of the microviscosity of cell membranes and their lipids in brain preparations from hibernating and warm-adapted hamsters substantiate the importance of membrane lipids in determining the physical properties of the membrane.

The Laboratory of Perinatal Physiology has continued to evaluate the ability of anesthetics to modify deleterious effects of asphyxia on the fetal monkey brain. Although high dose levels of pentobarbital may cause asphyxia by venous pooling and resultant maternal hypotension, moderate dose anesthesia actually improves oxygenation of the asphyxiated fetus, and thus may be of therapeutic value. Other investigative efforts have dealt with the pathologic effects of hypotension produced by ganglionic blocking agents. Measurements of regional cerebral blood flow during arterial hypotension by both thermal dilution and autoradiographic techniques indicate that a marked depression of

blood pressure leads to a generalized diminution in flow throughout the cerebral tissues without significant regional variations. These results fail to support the widely accepted "border zone" hypothesis of brain injury during hypotension.

In the Laboratory of Biophysics, recent experiments on ion interactions in open potassium channels of squid giant axon membrane have provided new information concerning the energy barriers crossed by ions entering these channels, and about the nature of ion binding sites within the channels. "Inertia" previously seen in potassium channels following large, brief depolarizations was confirmed and calculations performed to examine predictions of the Hodgkin-Huxley model under similar circumstances. These calculations indicate a qualitative divergence between experimental results and Hodgkin-Huxley predictions. The experimental observations were, however, consistent with predictions of the Baumann-Mueller model. Other laboratory elements have extended studies of mechanisms by which simple neural networks process and store information on a cellular level. A cell by cell analysis of the visual and vestibular pathways of the Hermissenda crassicornia has, for example, demonstrated the dominant role of presynaptic inhibition in producing persistent changes in network function.

The Laboratory of Central Nervous Systems studies has continued its pioneering work with slow, latent, and temperate virus infections of the nervous system. The successful demonstration that Kuru, a progressive degenerative disorder of the central nervous system, is caused by a serially transmissible virus possessing unconventional biologic and biochemical properties yielded the necessary techniques for the subsequent discovery of a virus etiology for several other cerebral degenerative disorders including Creutzfeldt-Jakob disease, familial Alzheimer's disease and progressive supranuclear palsy. These discoveries have also enabled recognition that Creutzfeldt-Jakob disease may be transmitted by transplantation surgery, warranting concern for possible contamination of tissue transplantations from donors with dementia or other neuronal degenerative conditions. It has also been possible to identify the virus of transmissible virus dementia (CJD-type) as the cause of death of a neurosurgeon, raising the possibility of occupational hazard. Recent demonstration of the transmissibility of scrapie from American sheep and English goats to several species of nonhuman primates, producing in monkeys a disorder which is indistinguishable from transmissible virus dementia of man, suggests a close relationship between scrapie and human spongiform encephalopathies.

A large foci of epilepsy in the Wissel Lakes area of West New Guinea has been found to result from cysticercosis with larvae of Taenia solium, a pig-tapeworm, newly introduced into New Guinea. In contrast to previous beliefs, it now appears that convulsions are due not to death of the worm with resultant scarring and calcification, but rather to acute inflammation from new invasion of the brain by Taenia larvae.

The Laboratory of Neurophysiology has continued to study properties of neuronal membranes, synapses, and receptors. By applying voltage clamp techniques to molluscan neurons, monovalent and divalent cation conductances have been measured in neurons which display pacemaker or bursting activity. Comparative investi-

gations of fish visual pigments, using spectrophotometric techniques, have provided new interpretations of the absorption properties and evolutionary development of these substances. Retinal studies designed to increase understanding of photoreceptor mechanisms and provide clarification of the main principles of retinal cell organization have revealed new properties in the synaptic arrangement of bipolar and amacrine cells. Physiologic experiments on photoreceptors have helped to define the role of calcium and other ions in the transduction process. Other experiments have shown that rod and horizontal cells of Bufo Marinus often develop voltage oscillations, strongly suggesting the existence of reverberation pathways between these cell types.

The Laboratory of Neuropathology and Neuroanatomical Sciences has extended previous investigations into the pathophysiology of cerebral ischemia. Experiments in mongolian gerbils indicate that systemic hypertension during reestablishment of circulation following an ischemic period has a markedly deleterious effect on brain cell morphology and on the integrity of the blood brain barrier. The former appears due in large measure to the reduction in the transport out of brain of various toxic substances, including lactate. Ischemic brain edema, resulting from an increased permeability of the blood brain barrier, now appears to be attributable to: (1) increased vesicular transport across the endothelium and (2) mechanical disruption of the endothelium in necrotic foci.

Other research has concerned the effects of experimentally induced seizures on GABA and cyclic nucleotide levels in mouse brain. Some anticonvulsants, including dipropylacetic acid, increased GABA and decreased cyclic GMP in the cerebellum, while others, including phenobarbital and phenytoin, decreased cyclic GMP but had no effect on cerebellar GABA. During the period of increased GABA and decreased cyclic GMP, the threshold for electrically induced seizures was increased.

A new in vivo model has been developed using tadpole optic nerve to test the myelinotoxicity of biologic fluids from patients with various neurologic disorders. In double blind studies, CSF samples from 60% of patients with multiple sclerosis had positive myelinotoxic activity which tended to correlate with disease severity; no activity was found in 85% of CSF samples from control patients, suggesting that the tadpole optic nerve model may be useful for investigating mechanisms of demyelination in multiple sclerosis.

It is now firmly established that horseradish peroxidase readily crosses from muscle capillaries into axon terminals, and then migrates retrogradely to the nerve cell body. These findings indicate for the first time how circulating proteins, which are unable to penetrate the blood brain barrier, can gain entrance to central neurons having processes in contact with the peripheral circulation.

Using a new method for capturing fleeting structural details in functioning central synapses by combining rapid freezing with the freeze-fracture technique, it has been possible to show how synaptic vesicles fuse with the surface of the presynaptic membrane to discharge their contents. Components of the synaptic vesicle wall, incorporated into the surface membrane during synaptic activity have been found to be later recovered and incorporated into new

synaptic vesicles. The freeze-fracture technique has also revealed that particulate structures, thought to be receptor molecules within the postsynaptic membrane, may be specific for different types of synapses.

The Laboratory of Neuropharmacology has in its second year of operation continued basic and clinically applied studies of central synaptic mechanisms. Recent observations indicate that glutamic acid decarboxylase, the rate limiting enzyme in GABA synthesis, may in vivo be no more than 60% saturated with its cofactor, pyridoxal phosphate, and that certain endogenous substances may reduce this binding to 40% or less. Accordingly, glutamic acid decarboxylase activity may be regulated, at least partially, by factors which limit the availability of pyridoxal phosphate to the apoenzyme. Related experiments have provided some basis for the possibility that pharmacologically manipulatable differences exist between GABA-containing neurons comprising striatal efferent systems and other GABA-containing neuronal pathways. This possibility derives from the finding that substantial regional differences exist in GABA turnover and that brain regions with faster rates of GABA turnover are much more sensitive to the effects of drugs that diminish GABA synthesis.

Clinical studies of a new class of dopamine agonists now indicate that the ergot derivative, bromocriptine, exceeds L-dopa in therapeutic efficacy in some patients with idiopathic Parkinson's disease. Bromocriptine has been found to be particularly useful in ameliorating "on-off" phenomena, a major complication in the long-term treatment of parkinsonian patients with L-dopa. Attempts to ameliorate choreatic movements in patients with Huntington's disease through the administration of a centrally active cholinergic agonist, pilocarpine, were unsuccessful. Since pilocarpine is a relatively weak stimulator of postsynaptic cholinergic receptors, however, these results cannot be construed as ruling out the possibility that diminished striatal choline acetyltransferase activity in Huntington's chorea patients reflects degeneration of striatal neurons which use acetylcholine as their neurotransmitter, and therefore that pharmacologic attempts to restore cholinergic function might afford symptomatic relief.

Studies on the pathogenesis of lead neurotoxicity have focused on the role of central dopaminergic mechanisms. Incubation of brain minces with lead significantly increased dopamine release and inhibited its reuptake, thus possibly explaining the increased urinary excretion of catecholamine metabolites found in children exposed to lead.

The Medical Neurology Branch has continued to apply basic research techniques to clinical problems involving the neuromuscular system. New methods have been found to detect early subcutaneous calcifications, which may later become a disabling complication of childhood dermatomyositis, using either ^{99m}Tc-di-phosphonate body scanning or xerography. By these techniques a remarkable diminution in calcium could be demonstrated as patients responded to combined azathioprine plus high single dose, alternate day prednisone therapy.

The laboratory has developed a new model of Duchenne muscular dystrophy. It involves the focal application of a mitochondrial poison to muscle fibers in situ, resulting in a secondary loss of the integrity of the sarcolemmal mem-

branes of the damaged fibers, and thus establishes an experimental basis for the hypothesis that the human disorder reflects a primary defect in the energy source within muscle fibers. Although the possibility of an ischemic mechanism for Duchenne dystrophy has yet to be directly demonstrated, it has now been shown that the pattern of histochemopathology in human muscle disease due to vascular occlusion overlaps that of early Duchenne dystrophy as well as with that of the laboratory's experimental ischemic myopathy in animals.

Last year the laboratory reported in sera of myasthenia gravis patients a newly recognized IgG which blocks the binding of alpha bungarotoxin to human acetylcholine receptors both at the normal neuromuscular junction and at extra-junctional receptors of denervated fibers. Recent studies indicate that all myasthenia patients having an IgG antimuscle antibody also have this blocking factor, although only half with blocking factor also had the antimuscle antibody. The junctional localization of the blocking factor is appropriate to impair neuromuscular transmission and cause the characteristic weakness of myasthenia gravis. Blocking factor positive myasthenia gravis sera has now also been shown to inhibit the binding of alpha bungarotoxin to the diffuse extrajunctional receptors of aneurally cultured human or animal muscle fibers. The multifactorial nature of the disimmune phenomena in myasthenia gravis was reemphasized by the laboratory's finding of an antinative-DNA antibody in the sera of a significant number of myasthenic patients.

The Surgical Neurology Branch has continued laboratory and clinically applied investigations of brain tumors. Studies in a murine glioma model showed that significant increases in survival time occurred with preimmunization but not with immunotherapy using standard subcutaneous immunization techniques. Chemotherapy with CCNU in the same model also increased survival time, and the effects of preimmunization and CCNU were additive. A review of clinical experience in more than 100 patients with cerebral gliomas revealed that a combination of surgical resection, radiotherapy, and chemotherapy with oral CCNU and intratumoral 8-azaguanine resulted in mean survival times for glioblastomas nearly twice those found with conventional therapy (surgery plus radiotherapy alone or plus radiotherapy and CCNU).

Clinical applications of computerized axial tomography have been further expanded. Studies on leukoencephalopathies, Huntington's disease, post-irradiation necrosis, and head injury are now in progress. The technique of computer assisted myelography using metrizamide has now been introduced. Work also continues on a new type of computerized tomography using a proton beam which may be five times more sensitive than current devices for detecting differences in physical properties of materials.

In the Developmental and Metabolic Neurology Branch, enzyme replacement therapy for inherited disorders remains a major focus of research interest. During the year, the therapeutic efficacy of exogenous glucocerebrosidase in patients with Gaucher's disease has been established, thus auguring well for enzyme replacement therapy for other heritable metabolic disorders. Of potentially great importance to enzyme replacement therapy is the recent observation that exogenous enzymes are taken up by the liver and that various agents, including pentobarbital, protect them against degradation. In other studies, a chromo-

genic analog of galactocerebroside has been successfully synthesized and shown to be a reliable reagent for the diagnosis of Krabbe's disease and for the identification of heterozygous carriers of this disorder. The assay is so facile that it can be reliably performed in general clinical chemistry laboratories. During the year, the laboratory has also demonstrated that gangliosides function as cell surface receptors for trophic hormones. For example, thyrotrophic hormone was found to react strongly with ganglioside G_{D1b}. Thyroid membranes contain this ganglioside and it may well serve as the natural receptor for the trophic hormone.

Characterization of mucopolysaccharides in urine of patients with Maroteaux-Lamy syndrome indicate that these glycosaminoglycans consist primarily of dermatan sulphate and chondroitin-4-sulphate. The molecular size of the excreted mucopolysaccharides presents a consistent pattern which will be useful for diagnosis of the syndrome and for monitoring enzyme replacement trials with sulphatase B.

Myelin-specific glycoproteins from the central and peripheral nervous systems have now been isolated and their carbohydrate composition determined. They appear to be highly sulphated, indicating an important functional aspect of their constitution. Central nervous system glycoprotein has been shown to be a surface component of myelin, and thus potentially susceptible to attachment of viruses and modification by virus-associated enzymes.

Studies by the Infectious Diseases Branch have shown that Venezuelan equine encephalitis virus produces porencephalic cysts and bilateral cataracts in rhesus monkeys infected in utero. These findings are in agreement with clinical reports from Central and South America and demonstrate the teratogenic potential of this virus. A survey of patients with subacute sclerosing panencephalitis (SSPE) in the United States showed a very significant association with HL-A type W29, indicating a genetic component may operate along with the measles virus in producing this disorder. Furthermore, brain cells taken from patients with SSPE were found to be destroyed by SSPE sera together with complement. Specific inhibitions of measles cellular immunity were found in the sera and CSF of patients with SSPE; CFS levels were approximately ten times higher than serum levels. These findings suggest that a "blocking factor" may contribute to the pathogenesis of this disorder. Electron microscopic studies of tissues infected with SSPE measles virus have shown defective virus budding, indicating that a specific defect in virus maturation occurs with this chronic virus infection.

The Neuroimmunology Branch, created two years ago, has begun investigations of the mechanisms by which the interaction between viruses and the host immune system either facilitates or inhibits the development of acute or chronic viral infections. Because certain central nervous system disorders may be related to an immunologic attack on myelin, elucidation of its molecular organization, especially with respect to the outer membrane surface, has been undertaken. Available evidence now indicates that a glycoprotein is a major molecular component of the outer membrane surface of myelin. The laboratory has also initiated studies of effector mechanisms operative in experimental allergic encephalomyelitis, and of immune function in patients with multiple sclerosis. Results of

the latter investigations have confirmed an increased representation of HLA-A3, HLA-B7 and HLA-DW2 histocompatibility antigens.

The Laboratory of Experimental Neurology has found further evidence suggesting that neuronal pathways mediating focal paroxysmal activity shift from subcortical to cortical structures with maturation. A search is now underway for subcortical circuits and nuclear masses involved in the propagation of seizure activity. Studies of the delayed effects of X-irradiation on the brains of adult monkeys have been extended to more closely simulate the clinical situation. Primates subjected to daily supervoltage irradiation, bracketing the usual human dose, developed small, widely scattered necrotic lesions, with a predilection for the forebrain white matter. Subsequently, the lesions became confluent and associated with brain swelling. Not only was there a delay of weeks or months before onset of overt tissue breakdown, but the individual lesions evidently appeared at different times, thus further protracting the period of their clinical influence.

The Clinical Neurosciences Branch has studied the effects of chronic, unilateral alternating or bilateral cerebellar stimulation in epileptic patients: both the frequency of spontaneous seizures and the requirement for anticonvulsant drugs appeared to be reduced. Chronic cerebellar stimulation was also associated with a significant elevation in CSF levels of norepinephrine. Interestingly, stimulation of the caudate nucleus in patients undergoing thalamotomy for dyskinesia was followed by a substantial reduction in the CSF content of this neurotransmitter. In other studies, the mean density of Purkinje cells in the cerebella of epileptic patients was found to be less than 40% of that in control subjects who were free of neurologic disease. In all cases, Purkinje cell loss was accompanied by isomorphic gliosis.

The Laboratory of Neuro-otolaryngology, one of the Intramural Program's more recently formed research units, has initiated investigations into the morphology and biochemistry of the inner ear. This work has resulted in the isolation and purification of a major cochlear enzyme, a carbonic anhydrase, whose high concentration in the cochlear membrane wall suggests that it may play an important role in maintaining electrolyte concentrations in the endolymphatic fluid. Other investigations have provided support for the hypothesis that olivocochlear neurons are cholinergic. On the other hand, available results now indicate that neither acetylcholine, GABA, nor catecholamines serve as major neurotransmitters for the auditory nerve in the cochlear nucleus. Electron microscopic studies of membranes of auditory sensory neurons and adjoining supporting cells using thin sections and freeze-fracture techniques have revealed tight junctions which may form the structural basis for the ionic concentration differences between endolymph and perilymph, known to be necessary for auditory function. Using similar electron microscopic techniques, synapses of the organ of Corti were studied. Both pre- and postsynaptic membrane structures at inner hair cells were different from those at outer hair cells, providing new evidence for the possibility that different hair cells may use different transmitter substances.

Annual Report of the Section on Technical Development

National Institute of Mental Health

National Institute of Neurological and Communicative Disorders and Stroke

July 1, 1975 - June 30, 1976

Theodore R. Colburn, Ph.D., Chief

The Section on Technical Development is a group of engineers, computer specialists, and technicians which provides technical services to the Intramural Research Programs of NIMH and NINCDS. The major functions of the Section are:

(1) Instrumentation research and development. Design and development of instruments and instrumentation systems which represent advances in the state-of-the-art. Most of the research within the Section falls in this category, and is generally done in collaboration with investigators in the laboratories of NIMH and NINCDS.

(2) Production of custom instrumentation. Design and fabrication of electronic, mechanical, and optical equipment to suit the particular needs of the requesting investigator. These instruments, while often quite complex, utilize rather than advance the current state-of-the-art in design techniques and components.

(3) Computer services. The Section assists the investigators in data collection, reduction, and analysis, by supporting two laboratory digital computers for general use, including real-time on-line applications, and by providing programming service and technical consultation.

Additional services provided by the Section include consultation on: measurement techniques, signal processing; noise and electro-magnetic interference in data measurement systems; and equipment purchases. Several formal and informal courses for investigators are taught by Section personnel; topics include electrical circuit theory, operational amplifier applications, digital logic design, and computer applications.

Due to manpower limitations and economic considerations, the Section is unable to provide the following services: repair of commercial instruments, duplication of off-the-shelf commercially available equipment, and fabrication of non-instrument items (shelves, bookcases, etc.).

INSTRUMENTATION

The following are selected instrumentation projects undertaken during the past year. These are chosen from a total of 233 projects, and are representative of the types of electronic instruments and systems developed by the Section.

(1) Patient Activity Monitoring System. The Section began last year, and completed this year, development of a wearable device to continuously monitor the movement activity of an ambulatory human subject and record the data in a solid state memory internal to the device. A piezoelectric accelerometer generates a voltage as the subject moves; when the voltage exceeds a preset threshold, a pulse is generated. The pulses, defined as activity units, are counted during a standard time interval (e.g. 15 min.). At the end of the interval, the total count is stored in memory, and a new count initiated. The memory can store 256 counts, each of which is 0-3072 activity units. Thus, if the counting interval is 15 min., a 64 hour profile of activity can be stored in the device. The counting interval may be selected as 3.75, 7.5, 15, 30, or 60 min; other intervals are possible but not used in practice.

More than twenty monitors are now in the field, used by six different research groups. Applications include research on manic-depressives, hyperactive children, drug addicts, subjects on special nutrition diets, and restrained monkeys. The parts cost of each device is \$120. It is housed in a stainless steel case, 1.5 x 4.1 x 6.3 cm, and is attached to a limb with a velcro strap.

Simple interfaces between the patient activity monitors and the Section's two PDP-11/40 computers were developed. With the computer handling the read-out, permanent storage, numerical processing, and plotting of the activity data, the need for manual data handling has been essentially eliminated. This development has been a major factor in the success of the activity monitoring programs thus far.

Each group that is using the activity monitors has been provided with a small device that is used to reset the internal timer and clear the memory of each monitor just prior to application of the monitor to the patient. With this device, the reset and clear operations can be made independent of the read-out computers.

An electro-mechanical calibration device has been developed for quantitative characterization of each activity monitor's sensitivity to simulated patient movement. The speed of a DC motor determines the amplitude and frequency of acceleration that two monitors simultaneously undergo. The motor speed is under programmed logical control so that acceleration sequences can be accurately repeated.

(2) Isolated Iontophoresis Current Source. The primary feature of this instrument is the ability to pass an iontophoretic current between two intracellular micropipettes while minimizing any current flow to ground in order to avoid electrical stimulation of the cell. For increased versatility, the instrument also includes a ground-referenced current source and a capacitance neutralized voltage recording amplifier. This combination

permits measurement of cell membrane conductance changes due to intracellular drug injections.

(3) Multi-Channel Iontophoresis Current Sources. This instrument has been further refined since its design last year. The unique current monitor has been improved to give an accurate reading even when the micro-pipette becomes partially or completely blocked. This should prevent false negative findings of drug efficacy and also permits the inclusion of an unbalance current monitor. This device indicates when the difference between the total drug current from the three pumping channels and the current from the balance channel exceeds a preset unbalance level. In addition, the application of the drug current for each of the three channels may be synchronously controlled by a digital gate from an external timing device. This facilitates both accurate repetitive drug application and computer analysis of neuronal responses.

(4) Eye Movement Monitor. An instrument is being developed to monitor horizontal eye position in monkeys. A beam of infrared light from a gallium arsenide diode is directed toward the center of the cornea. The reflected light is sensed by two matched phototransistors positioned on either side of the cornea and directed toward the center of the eye over the iris-cornea boundary. As the eye turns, the bulge of the cornea causes a variation in the amount of infrared light reflected to the two phototransistors. The transistors convert light intensity to voltages which are differentially amplified to produce an output voltage representative of horizontal eye position. The three opto-electronic devices are rigidly mounted on the rim of a machined circle of nylon fitted to an aluminum framework, which allows the nylon eyepiece to be adjusted in three axes by means of small rack and pinion gears. In addition, the eyepiece can be rotated about its vertical axis and tilted toward or away from the eye. With these mechanical adjustments the electronics can be arranged for optimum linearity, balance and gain. At this time, the system is adequate for observing eye movements in human subjects. Although the skull shape and disposition of monkeys is somewhat different, the outlook for adapting the device to vision research in monkeys is quite promising.

(5) Measurement of Activity of Confined Animals. During the past year, the Section took on four separate projects to measure the movement activity of animals in various confined situations.

(a) Rat Activity Monitoring Cage. A unique type of cage is being developed to monitor the movement of unrestrained rats. The 30 x 30 cm floor is divided into nine areas, each 10 x 10 cm, constructed of electrically isolated metal grids. An audio frequency system employing solid-state switching and capacitive coupling detects when the animal is contacting two or more grids simultaneously, and determines which grids they are. Thus patterns of movement can be determined. A tenth grid around the sides of the cage, at an appropriate height, allows detection of when and where the animal rears. In addition, the cage floor is mounted on shock absorbers and equipped with accelerometers, as an additional measure of motion. Data will be processed and recorded using a microprocessor system.

(b) Activity Monitor for Restrained Rat. In order to detect movement of a rat which is restrained by confinement in a very small volume, a capacitive system was developed. Parallel metallic strips on the bottom of the cage form a capacitor, for which the rat is part of the dielectric. As the rat moves, the capacitance changes, thereby modulating the frequency of an oscillator. The frequency change is demodulated using a phase-locked loop, and the output voltage is proportional to movement. Common movement of both rat and cage is rejected by this system.

(c) Rat Rotameter. The clockwise and counter-clockwise rotations of a rat responding to drug treatment are measured by harnessing the animal to an overhead rotating connector which permits relatively free movement. CW and CCW rotations are measured via photo-isolators, the total count is stored for a certain time interval, and the total interval count is converted to analog signal for output to a strip-chart recorder.

(d) Activity of Monkeys in Restraining Chairs. A preliminary study of the patterns of activity of monkeys confined in restraining chairs is being carried out by simply affixing a Patient Activity Monitor to the monkey's head and reading the activity data every two days. The system ultimately to be developed will involve simultaneous on-line monitoring of activity and internal temperature, with data logging controlled by a microprocessor.

(6) Microprocessor Applications. A program to explore LSI technology in the form of microprocessors was undertaken to evaluate the possibility of applying the processing and control capabilities of these relatively inexpensive devices towards the types of instrumentation designed by this Section. A survey in the form of literature reviews and manufacturers seminars on microprocessor specifications, techniques and applications, led to the purchase of a microprocessor for evaluation. Results of the evaluation demonstrated numerous potential applications in the areas of interest.

Two typical examples are:

(a) Data Acquisition System. A microprocessor based system for continuous monitoring of activity from 16 rat exercise wheels was developed. One full rotation of a wheel sets an appropriate flag bit; every ten seconds the microprocessor scans all 16 flag bits and stores the data on a digital tape cassette. Data analysis of the activity patterns is done off-line on the Section's PDP-11/40 computer.

(b) Preprocessor for Movement Detector. A microprocessor will be used to preprocess and record the data from the Rat Activity Monitoring Cage described previously. The microprocessor will determine when the animal moves from one grid to another, and will record these events, along with integrated cage floor acceleration data, on a digital tape cassette.

(7) Condition Timer. A programmable timer was designed and constructed for an ongoing study of mother-child learning behavior. The device is used to time sequences of experimental conditions with a short "time-out" interval between conditions. The instrument allows the control of two experimental modes; the interval of time in either mode is selectable between 1 and 9,999 seconds. A four digit display shows the time remaining in the present mode. Between modes a "time-out" interval is also selectable

between 0 and 9 seconds. Eight mode select switches allow the instrument to be pre-programmed for an experimental session. The instrument provides a complete manual control if desired.

(8) AR-11 Interface Panels. Two interface panels were built for the PDP-11/10 computers to allow extended use of the computer's A to D, D to A, and scope control capabilities. The interface allows the user easy access to the 16 channels of A to D provided by the AR-11, and also allows the use of some of the digital control lines that are available for interfacing to certain display scopes. In addition, the interface includes a multi-use Schmitt-trigger, and circuitry necessary to modify the output control signals for use with existing displays.

(9) Isolated Current Monitor. An inexpensive current monitor has been developed which provides an output voltage proportional to but isolated from the input current. Photodiode optical isolators function in pairs to achieve the isolation and to compensate for the inherent non-linearities of current transfer in the isolators. When used in the return path of a "floating" iontophoresis system, the device maintains isolation between the iontophoretic current and the system recording cell responses, while providing a measure of the stimulating current. The instrument measures from .5nA to 5 μ A of current of either polarity in a bandwidth of DC to 20 KHz.

(10) Psychological Testing Apparatus. Special 8x8 cm. transparent panel switches were designed to mount in front of a computer graphic display terminal on which stimuli are presented. The transparent panels, which are inlaid in a larger opaque panel covering the terminal, are mounted such that when depressed, they activate photo-optical circuits interfaced to the computer. A different computer generated figure or shape is displayed behind each transparent panel. The subject makes a selection by depressing one switch; if the appropriate selection is made, a reward is dispensed by program control into a small container at the top of the display panel.

(11) Respiration Monitor. A respiration monitor for monkeys undergoing brain surgery was designed and constructed utilizing a thermistor bridge and A-C coupled operational amplifiers. Variable gain allows use for adult or infant animals. The principal output is a meter movement allowing qualitative assessment of the depth of breathing; however, alarm circuitry is included which provides an audio alert if a selected time interval elapses without respiration detection.

(12) Revolving Disc and Drive System. A 40 cm. diameter high inertia revolving disc was constructed to be used in the study of cells that respond to acceleration. A motor with sufficient torque and speed and a motor controller were purchased to drive the turntable. The controller was modified to provide a range of smooth accelerations and decelerations of the disc as well as an adjustable steady state speed.

COMPUTER SERVICES

In FY 1975, the Section on Technical Development initiated a survey of the computer needs of the Intramural Program and developed a plan for a modified distributed network consisting of small, inexpensive satellite computers in the laboratories supported by larger host computers in the major work areas. This year saw the delivery and installation of support computers in Buildings 10 and 36, and in the Animal Center in Poolesville. In addition, eight small computers were delivered to individual laboratories. The support computers in Buildings 10 and 36 are run by the Section, while the computer in Poolesville is run by the Laboratory of Brain Evolution and Behavior, with software support from STD. The Section oversaw the installation and initial running of the satellites.

Much of the programming effort was devoted to the development of general purpose routines specific to the needs of the Intramural Program and to study of the operating system and its utilization for neurophysiological and psychological applications. Assistance was provided to individual investigators for their specific applications. Examples are a high-speed digitization routine for subsequent analysis of the shape of evoked potentials and a remote installation for recording potential changes in the retina after light stimulation.

The installation and implementation of the new system has proceeded on schedule. The functions of the SEL 810B have been replaced and the use of that system will be terminated May 1, 1976. The microLINC 300 and PDP-12 have been transferred from the Section to perform dedicated applications in other laboratories.

STD offered three introductory courses in minicomputer structure, PDP-11 architecture and programming, and in the use of the operating system, RT-11. These courses were conducted in lieu of the courses offered, for a fee, by the manufacturers, and were specifically tailored for the user's systems. In addition, STD offers follow-up advice and consultation, services not offered by the manufacturer. STD also offers personalized instructions for individuals beginning to use the computers, and short courses in programming languages to small groups. STD personnel are available for advice and consultation at all times to the users.

The Section conducted systems studies for a number of individual laboratories, surveying their computer needs and offering a proposal for the procurement of an appropriate system. As a result of these studies, 2 additional satellites have been ordered in NIMH and a computer system for the analysis of autoradiographs of brain sections has been ordered for the Laboratory of Cerebral Metabolism. Pending available funds, the Behavioral Biology Branch of NICHD will purchase a satellite and the Division of Special Mental Health Research at St. Elizabeth's will purchase a support computer. STD also assisted the users of the satellites in determining the appropriate peripherals and scientific interfaces necessary for their experiments.

PL STD has been exploring the feasibility of using low cost, microcomputers as laboratory instruments. We simulated a prototype system on a support computer to read and store data from the Patient Activity Monitors for future analysis, resulting in the design and procurement of a microcomputer configuration for this purpose. STD is also conducting a study for the feasibility of low cost data acquisition systems for physiological data. Applications utilizing microcomputers and microprocessors are being evaluated by STD to see which will be more advantageous for specific applications. The Section is deeply involved in all areas utilizing the computer as a laboratory instrument and provides technical support and assistance in all phases of development.

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ENGINEERING, COMPUTER and FABRICATION SERVICES

This table shows the distribution of the Section's workload among the various laboratories.

<u>LABORATORY OR BRANCH</u>	<u>HOURS</u>	<u>PERCENT</u>
Adult Psychiatry Branch, NIMH - - - - -	3150	13.15
Neurophysiology, NINCDS - - - - -	2770	10.92
Biophysics, NINCDS - - - - -	1882	7.50
Behavioral Biology, NICHD - - - - -	1839	7.27
Neurophysiology, NIMH - - - - -	1698	7.08
Psychology & Psychopathology, NIMH - - - - -	1614	6.73
Special Mental Health Research, St. E's, NIMH - - - - -	1567	6.53
Clinical Science, NIMH - - - - -	1501	6.26
Brain Evolution & Behavior, NIMH - - - - -	1488	6.20
Developmental Psychology, NIMH - - - - -	1386	5.68
Molecular Biology, NINCDS - - - - -	1063	4.43
Equal Employment Opportunity, NIMH - - - - -	965	4.02
Neuro-Otolaryngology, NINCDS - - - - -	749	3.01
Neuropharmacology, NINCDS - - - - -	623	2.60
Neurobiology, NIMH - - - - -	488	2.03
Neurochemistry, NINCDS - - - - -	445	1.85
Neuropathology & Neuroanatomical Sciences, NINCDS - - - - -	264	1.10
Neurochemistry, NIMH - - - - -	246	1.02
Cerebral Metabolism, NIMH - - - - -	239	.99
Experimental Neurology, NINCDS - - - - -	168	.70
General & Comparative Biochemistry, NIMH - - - - -	166	.69
Medical Neurology, NINCDS - - - - -	126	.53
Intramural Research Program, NINCDS - - - - -	122	.508
Neuro-Immunology, NINCDS - - - - -	45	.0018
Surgical Neurology, NINCDS - - - - -	34	.0014

NIMH (Total)*	16,858	58.86
NINCDS (Total)*	9,648	33.68
NICHD (Total)*#	2,137	7.46
TOTAL	28,643	100.00

*These figures include administrative time not shown in the above listing.

#NICHD loans the Section one position, and is thus entitled to 2000 hours of service.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 00201 - 21 ODIR																																
PERIOD COVERED July 1, 1975 - June 30, 1976																																		
TITLE OF PROJECT (80 characters or less) Guam Research Center Studies																																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																																		
<table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">Principal Investigator:</td> <td style="width: 30%;">T. N. Chase</td> <td style="width: 20%;">Director</td> <td style="width: 20%;">IRP, NINCDS</td> </tr> <tr> <td>Other Investigators:</td> <td>D. C. Gajdusek</td> <td>Chief</td> <td>CNSS, NINCDS</td> </tr> <tr> <td></td> <td>C. J. Gibbs, Jr.</td> <td>Microbiologist</td> <td>CNSS, NINCDS</td> </tr> <tr> <td></td> <td>P. M. Hoffman</td> <td>Research Associate</td> <td>OD, NINCDS</td> </tr> <tr> <td></td> <td>K. M. Chen</td> <td>Consultant</td> <td>OD, NINCDS</td> </tr> <tr> <td></td> <td>O. Cruz</td> <td>Consultant</td> <td>OD, NINCDS</td> </tr> <tr> <td></td> <td>Hideki Igisu</td> <td>Guest Worker</td> <td>OD, NINCDS</td> </tr> <tr> <td></td> <td>Yasuho Nagano</td> <td>Guest Worker</td> <td>OD, NINCDS</td> </tr> </table>			Principal Investigator:	T. N. Chase	Director	IRP, NINCDS	Other Investigators:	D. C. Gajdusek	Chief	CNSS, NINCDS		C. J. Gibbs, Jr.	Microbiologist	CNSS, NINCDS		P. M. Hoffman	Research Associate	OD, NINCDS		K. M. Chen	Consultant	OD, NINCDS		O. Cruz	Consultant	OD, NINCDS		Hideki Igisu	Guest Worker	OD, NINCDS		Yasuho Nagano	Guest Worker	OD, NINCDS
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	Hideki Igisu	Guest Worker	OD, NINCDS																															
	Yasuho Nagano	Guest Worker	OD, NINCDS																															
COOPERATING UNITS (if any) Lab. of Central Nervous System Studies, IRP, NINCDS, Lab. of Neuropharmacology, IRP, NINCDS, Brain Research Institute, Niigata Univ., Niigata, Japan, Neurological Institute, Kyushu Univ., Fukuoka, Japan, Guam Memorial Hospital, Agana, Guam, University of Connecticut, Farmington, Conn.																																		
LAB/BRANCH Office of Director, IRP																																		
SECTION Guam Research Center																																		
INSTITUTE AND LOCATION NINCDS, Agana, Guam																																		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																																
8	3	5																																
SUMMARY OF WORK (200 words or less - underline keywords)																																		
<p> <u>Guam</u> Research Center is concerned with studying etiologic factors, pathogenetic mechanisms and therapeutic approaches to Guamanian <u>amyotrophic lateral sclerosis</u> (ALS) and <u>parkinsonism-dementia</u> (PD). Current projects include: Maintenance of a tissue bank in Bethesda; virologic studies including the search for a possible <u>C-type virus</u> particle; a detailed assessment of <u>immune function</u> including <u>HL-A Typing</u>, enumeration of circulating T-cells by the Rosette Method, the presence of auto-antibodies, lymphocyte transformation to phytohemagglutinin and several common antigens such as PPD, Candida, and Streptokinase-Streptodornase as well as mediator production (LI); and study of the long term effects of <u>L-dopa</u> in PD patients. </p>																																		

Objectives: To study etiologic factors, pathogenetic mechanisms and therapeutic approaches to Guamanian amyotrophic lateral sclerosis (ALS) and parkinsonism-dementia (PD).

Methods employed: Available epidemiological, neuropathological, neuropharmacologic, virologic and immunologic techniques are employed. Special emphasis was placed this year on methods for HLA typing, immunoglobulin measurement, both in vitro and in vivo approaches to the study of cellular immunity, and tissue culture.

Major findings: 1. HLA typing was performed on 60 PD patients, 23 ALS patients and 67 controls. A higher percentage of W16 (HLF -W39), a second locus antigen, was seen in the PD group (25%) as compared to controls (7%) but the difference was only of borderline significance. ALS patients had an intermediate percentage (15%). The population in general showed a distribution of antigens commonly associated with oriental populations with a high preponderance of HLA -- 2, 9, 10, W10, and W5.

2. Immunoglobulin levels were measured on 57 normal Guamanians, 34 ALS patients and 61 PD patients. IgA levels were significantly higher in PD patients than in controls while IgM levels were significantly lower than control in PD. ALS cases generally showed higher levels of all three immunoglobulins than controls, but not significantly so.

3. Diminished cellular immunity as measured by both in vitro and in vivo parameters exists in both ALS and PD patients, although it is more striking in PD. Enumeration of the T cells by the Rosette method showed that both PD and ALS patients had values which were significantly lower than age-matched controls of the percentage of circulating lymphocytes that carried the marker and of total number of circulating T cells.

4. In cooperation with Laboratory of Central Nervous System Studies, diseased and normal brain and skin cells were grown and reproduced in culture. No cytotoxic effects have been observed thus far, but the cultures are being stored in Bethesda for future investigations.

5. Virologic studies in collaboration with Dr. Michael Viola, University of Connecticut, and Dr. Lon White, Laboratory of Central Nervous System Studies, continue to explore the possibility that a C-type virus particle is present in Guamanian tissues. The major techniques being applied to this study are detection and characterization of the oncornovirus-like reverse transcriptase; and use of nucleic acid hybridization to identify viral RNA or DNA in brain cells. Steps are being taken to obtain sufficient quantities of enzyme to perform the necessary biochemical and immunological procedures for complete characterization of the particle.

6. A tissue bank of frozen and fixed brains, spinal cords, spinal fluid, sera and various internal organs is now being maintained in collaboration with the Laboratory of Central Nervous System Studies. The inventory is currently being computerized making specimens more readily available to collaborating

7. Studies continue on the long-term therapeutic and toxic effects of L-dopa in combination with a peripheral decarboxylase inhibitor in PD patients. In addition, the effects on longevity of this treatment are being evaluated.

8. Computerized registries of all PD and ALS cases as well as controls continue to be maintained both on Guam and in Bethesda.

9. A health survey was performed of Umatac, the area of highest incidence of PD and ALS. Approximately 210 people were examined but only one probable and several possible cases of PD were discovered. These cases will be followed.

10. Genetic analysis of family data on ALS and PD in Umatac has been compiled by the Center for Demographic and Population Genetics, University of Texas at Houston.

11. The Research Center continues to provide diagnosis, follow-up and treatment of patients on Guam as well as surrounding islands.

Significance to biomedical research and the program of the Institute: Guam has the highest incidence in the world of motor neuron disease and the unique disease PD. Documentation of the epidemiological, clinical, and neuropathological aspects of ALS and PD have contributed to our knowledge of related central nervous system degenerative diseases. In fields having no known causes or cures, data such as these provide one of the most likely avenues for development of concepts and facts which lead to prevention and treatment.

Proposed course: Immunologic clues established this year will be pursued in greater detail. The search for a C-type virus will continue as a collaborative study between the Laboratory of Central Nervous System Studies and the University of Connecticut.

Publications: Viola M., Frazier M., White L., Brody J. and Spiegelman S.: RNA-Instructed NDA Polymerase Activity in a Cytoplasmic Particulate Fraction in Brains from Guamanian Patients, The Journal of Experimental Medicine, 145:483-494, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS-01526-09-ODIR
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PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Epileptic Neurons and Their Recurrent Axon Collaterals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. L. Li	Associate Neurosurgeon	IRP, NINCDS
OTHER:	A. F. Bak	Electronics Engineer	LNP, NINCDS

COOPERATING UNITS (if any)

Laboratory of Neurophysiology, NINCDS

LAB/BRANCH

Office of the Director of Intramural Research

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

0.3

PROFESSIONAL:

0.2

OTHER:

0.1

SUMMARY OF WORK (200 words or less - underline keywords)

Passing negative electrical current into the interior of a nerve cell in the cortex produces hyperpolarization and inhibition of the cortical neuron. Inhibition is followed by post-inhibitory depolarization and discharges. Conversely, passing positive electrical current into the interior of a cortical neuron produces depolarization and excitation of the neuron followed by post-excitatory hyperpolarization. High frequency depolarizing pulses applied to a neuron does not produce after-discharges but a prolonged post-excitatory hyperpolarization of the cell membrane.

Project Description:

Objective: To study the electrophysiological properties of the individual nerve cells in the cerebral cortex under different experimental conditions.

Methods Employed: Glass micropipette electrodes were inserted into the cells in the motor cortex of anesthetized cats. The electrical activity of the cells was recorded; and, thereafter, pulses of electrical current of increasing intensity and of different frequencies were applied through the intracellular recording electrode. The electrical resistance of the cell was derived from the current-voltage ratio and the time constant of the cell membrane and capacitance were calculated. This was carried out before and after the application of strychnine to the exposed cortex. In some experiments, repetitive electrical stimulation was also applied to the surface of the cortex through a gross electrode adjacent to the recording micropipette electrode, or to the individual cells through the recording micropipette electrode.

Major Findings: Some of the electrical properties of cortical neurons have been reported previously. The preliminary observations suggest that they are different from neurons under the effect of strychnine. For instance, the resistivity of normal cortical neurons measures $(9.17 \pm 2.68) \times 10^6$ ohms while that of the epileptic neuron was found to be in the order of 5 to 6×10^6 ohms. Further, epileptiform activity of the cortex requires "synchronous" presynaptic bombardments and there is good evidence of negative feedback through the axon collaterals to the epileptic neurons. Finally, the hypothesis or theory of Burns (1962) was tested. According to Burns' theory, repetitive discharges of a single cortical neuron would produce after-discharges which spread in all directions; thereby precipitating local epileptiform activity of the cortex. The present study proves that this theory is not valid, apparently due to the powerful negative feedback system among the cortical neurons. Reports of these findings are in process.

Significance to Biomedical Research and the Program of the Institute: The electrical properties of the cell determine the cell's "natural" behavior as well as its response to the environment. If the electrical properties of the epileptic neurons are different from that of normal cortical neurons, it seems likely that any effective pharmacological treatment will restore the normal electrical properties. Similarly, the electrical measurements may serve as a criterion of the effectiveness of the treatment.

Proposed Course of Project: This project was terminated last year because of the fact that the principal investigator was fully occupied by a study in regard to acupuncture-analgesia. Since the acupuncture-analgesia project was discontinued, the present study is again re-activated with special emphasis in pharmacological treatment of epilepsy.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-01527-09-ODIR

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Physiological Mechanism of Motor Function in the Cat

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: C. L. Li

Associate Neurosurgeon

IRP, NINCDS

OTHER: None

COOPERATING UNITS (if any)

None

LAB/BRANCH

Office of the Director, Intramural Research

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

0.3

PROFESSIONAL:

0.3

OTHER:

0.0

SUMMARY OF WORK (200 words or less - underline keywords)

When the dorsal portion of the caudate nucleus was anodally polarized in reference to the ventral portion, the neurons in the anterior sigmoid gyrus were found to be depolarized; whereas when the dorsal portion was cathodally polarized, the neurons became hyperpolarized. The hyperpolarization response of the cortical neurons has a higher threshold than the depolarization response.

Project Description:

Objective: To study the functional organization of the pyramidal and extrapyramidal pathways.

Methods Employed: Intracellular and extracellular micropipette electrodes were used to record the activity of the nerve cells in the motor cortex of the cat and stimulation was applied to the basal nuclear structures. In the past year stimulation was applied primarily to the caudate nucleus and nucleus ventralis lateralis of the thalamus.

Major Findings: The discharges of cells in the motor cortex increase or decrease and the membrane potential increases or decreases in response to polarization of the ipsilateral caudate nucleus.

Significance to Biomedical Research and the Program of the Institute: This study provides further information about the interaction of the pyramidal and extrapyramidal system at the central level. The present investigator is aware, however, that the regulatory effect of the extrapyramidal system on motor function may be found in the cells of the motor cortex itself because 4-5% of the cells in the motor cortex have descending axons in the medullary pyramid. He is also aware that one of the causes of involuntary movements is due to metabolic disturbances of the subcortical basal structures and that pharmaceutical treatment of the abnormal movements is directed to the metabolic activity of the basal structures. The extension of the present study may add to our understanding of the function or dysfunction of motor movement and of the physiological action of some of the drugs in the treatment of Parkinson's disease.

Proposed Course of Project: This project was terminated last academic year because of the fact that the present investigator was fully occupied by a study in regard to acupuncture-analgesia. Since the program of acupuncture-analgesia was discontinued, the present investigator had more time to renew his past interest in the study of motor function at the cellular level. It is anticipated that further experiments will be carried out with particular reference to the effect of pharmacological agents on the subcortical basal structures.

Publications:

Li, C.L. and Ratcheson, R.A.: Polarization of caudate nucleus and excitability of neurons in motor sensory cortex. Exp. Neurol. 50: 134-145, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02009-04-ODIR

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Conditioning of Pain by Acupuncture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	C. L. Li	Associate Neurosurgeon	IRP, NINCDS
OTHER:	H. Lansdell	Psychologist	FNP, NINCDS
	A. F. Bak	Electronics Engineer	LNP, NINCDS
	M. Gravitz	Psychologist (Consultant)	IRP, NINCDS
	C. Y. Ting	Acupuncturist (Consultant)	IRP, NINCDS
	D. Blessing	Registered Nurse	VR, DRS

COOPERATING UNITS (if any)

Fundamental Neuroscience Program, NINCDS; Laboratory of Neurophysiology, NINCDS; and Veterinary Resources Branch, Division of Research Services.

LAB/BRANCH

Office of the Director, Intramural Research
SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

0.0

PROFESSIONAL:

0.0

OTHER:

0.0

SUMMARY OF WORK (200 words or less - underline keywords)

Acupuncture and hypnosis were given in separated sessions to healthy volunteers. Before, during and after induction of acupuncture or hypnosis, painful electrical stimuli were applied to the supraorbital branch of the right trigeminal nerve. During this time blood samples and electroencephalograms were taken. It was found that acupuncture does not reduce the pain sensation while hypnosis does. Finally, under acupuncture treatment there is no change in the blood chemistry and brain waves.

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS-02010-04-ODIR												
PERIOD COVERED July 1, 1975 to June 30, 1976														
TITLE OF PROJECT (80 characters or less) Neurophysiological Mechanisms of Pain														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">C. L. Li</td> <td style="width: 35%;">Associate Neurosurgeon</td> <td style="width: 15%;">IRP, NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>G. Mathews</td> <td>Neurosurgeon</td> <td>V.A. Hospital, Washington, D.C.</td> </tr> <tr> <td></td> <td>A. F. Bak</td> <td>Electronics Engineer</td> <td>LNP, NINCDS</td> </tr> </table>			PI:	C. L. Li	Associate Neurosurgeon	IRP, NINCDS	OTHER:	G. Mathews	Neurosurgeon	V.A. Hospital, Washington, D.C.		A. F. Bak	Electronics Engineer	LNP, NINCDS
PI:	C. L. Li	Associate Neurosurgeon	IRP, NINCDS											
OTHER:	G. Mathews	Neurosurgeon	V.A. Hospital, Washington, D.C.											
	A. F. Bak	Electronics Engineer	LNP, NINCDS											
COOPERATING UNITS (if any) Veterans Administration Hospital, Washington, D.C. and Laboratory of Neurophysiology, NINCDS														
LAB/BRANCH Office of the Director, Intramural Research														
SECTION														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014														
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.3	OTHER: 0.1												
SUMMARY OF WORK (200 words or less - underline keywords) <p> Stimulation of the saphenous peripheral nerve with haversine wave electrical current selectively evoked C-fiber responses. With this technique, the <u>excitability characteristics</u> of the <u>A-</u> and <u>C-fibers</u> were determined. A similar experiment was carried out with sympathetic nerves. Finally, interaction of the impulses generated by A and C fibers at different levels by the central nervous system, and effects of different <u>pharmaceutical agents</u> on the C-fiber responses were investigated. </p>														

Project Description:

Objectives: To study the physiological mechanisms of pain and changes in pain responses to other sensory inputs and to pharmacological agents.

Methods Employed: The saphenous nerves, vagus and cervical sympathetic, of the cat were used. They were stimulated through a function generator with Haver-sine-wave electrical pulses of varying intensities and durations. This differentiates the C component and A-delta component from the other components of the responses initiated by the mixed nerve. The C and the A-delta component were also recorded with extracellular and intracellular electrodes in the central nervous system at different levels. Interaction of these responses with other responses initiated from the mixed sensory nerve fibers was studied. These responses were also studied under the influence of various analgesic agents.

Major Findings: We have developed a method to differentially activate the A or C fibers in a peripheral nerve. The C-fibers have a rheobase of 0.033 ma and chronaxy of 1.5 msec; the A-delta fibers have a rheobase of 0.0166 ma and chronaxy of 0.45 msec. The average conduction velocity of the A-beta fibers is 85.8 meters per sec., of the A-delta fibers, 11.2 meters per sec., and of the C-fibers, 0.92 meters per second. These findings indicate that the A-fibers have a threshold of 75 times as high as the C-fibers. The present study also discloses that there is a large component of C fibers in the vagus nerve.

Significance to Biomedical Research and the Program of the Institute: Needless to say, pain has a history as long as the human race, yet its mechanisms remain to be understood. Similarly, there are at least 154 pharmaceutical products which are known to alleviate pain, but their site of action is generally unknown. For example, it is still debatable whether sodium salicylate acts on the receptors, peripheral nerves or on the midbrain reticular formation even though the perivascular nerve fibers were claimed to be the site of action. The present investigation may contribute to our knowledge of pain and therapy of pain.

Proposed Course of Project: The present study is to be continued with particular emphasis on the cell activity and the electrical properties of the cell membranes in the central nervous system in response to pain and analgesic agents.

Publications:

Li, C.L. and Bak, A.: Excitability characteristics of the A- and C-Fibers in a peripheral nerve. Exp. Neurol. 50: 67-79, 1976.

Li, C.L., Ahlberg, D., Lansdell, H., Gravitz, M.A., Chen, T.C., Ting, C.Y., Bak, A.F. and Blessing, D.: Acupuncture and hypnosis: Effects on induced pain. Exp. Neurol. 49: 272-280, 1975.

Mathews, G. and Li, C.L.: Psychogenic pain: A critique. Pain (Journal of the International Association for the Study of Pain) (In Press).

Mathews, G. and Li, C.L.: Common Errors in Pain Management.

In Wittkower, E.D. (Ed.): A Psychosomatic Approach to Medical Practice. New York, Harper & Row. (In Press).

Annual Report
July 1, 1975 through June 30, 1976
Medical Neurology Branch, IRP
National Institute of Neurological and Communicative Disorders and Stroke

W. King Engel, M.D.
Chief, Medical Neurology Branch

Introduction: An inter-related multidimensional attack on the chosen target diseases is emphasized in our application of basic research techniques to clinical neurologic problems. The current techniques consist of: histochemistry, tissue culture, electronmicroscopy, immunology, autoradiography, biochemistry, and clinical neurophysiology. In the human neurologic disorders studied, these techniques support thrusts to seek: (a) more precise morphologic, electrical, immunologic and chemical definition of the abnormalities; (b) separation of each disorder into more distinct and often new sub-forms; (c) specific or symptomatic treatment; and (d) induced animal models closely related to the human pathophysiologic states. Our main emphasis is on the neuromuscular diseases -- they are considered to be affecting more than 1,000,000 persons in the country.

For the clinical investigations, 317 patients were admitted for a total of 7,962 patient days, and there were 1,135 outpatient visits. About 450 human muscle biopsies were processed histochemically and reported out -- about 100 of those were from outside hospitals. Many other outside biopsies are submitted for formal opinion. Neurologic consultations were provided on 490 patients of other departments in the Clinical Center, with performance of indicated muscle and nerve biopsies and electromyograms. In the past year, 19 papers were published, 34 are in press, and there were more than 60 presentations to meetings.

The one-year approved residency training program in neurology was continued. Approximately 12 neurologists and other physicians and 10 technicians came this past year to learn clinical research techniques in neurology, especially in neuromuscular diseases and the application of enzyme histochemistry thereto. A few residents from various other hospitals rotate through our service. The fourth annual 3-day course on neuromuscular diseases in Houston, in collaboration with the Veterans Administration and Baylor, was organized and most of the lectures given by our present and former staff. Four years ago we initiated (with IO-OD) the NINCDS videotape series of teaching lectures by putting the speakers of the first Houston symposium on tape -- more than 1,000 requests for those tapes have been filled.

Many of our former trainees are full professors, associate professors, and assistant professors in academic departments, and many are directors of Muscular Dystrophy Clinics and Myasthenia Gravis Clinics; many are Medical Advisory Board members of the national Muscular Dystrophy, Myasthenia Gravis, Amyotrophic Lateral Sclerosis, and Multiple Sclerosis Society/Association/Foundation.

A collaborative program on neuromuscular diseases with INSERM, Paris, through Hôpital Salpêtrière and Hôpital des Enfants Malades has been developed.

Myopathies are non-neurogenic, primary or secondary diseases of muscle. Some, such as the dermatomyositis/polymyositis group, are often at least partially treatable but their cause and details of their probably "dysimmune" pathogenesis are not known; others are not treatable but their cause is known, e.g., genetic deficiency of phosphorylase, phosphofructokinase, acid-maltase or carnitine-palmitoyl-transferase; while still others, such as Duchenne muscular dystrophy and others bearing the name dystrophy, are both of unknown pathogenesis and untreatable.

The polymyositis/dermatomyositis (PM/DM) disease-complex is an acquired disorder causing progressive deterioration of muscle in children and adults. The primary cause is not known but the pathogenic mechanism is considered "autoimmune" or "dysimmune". All patients were seriously incapacitated and many fatal before the introduction of anti-dysimmune therapy. Our studies involve improving diagnostic methods, seeking the cause and analyzing the pathogenic mechanisms, and improving methods of treatment.

The special variation of anti-dysimmune treatment we introduced to this disease 8 1/2 years ago, long-term high-single-dose alternate-day prednisone (LT-HSDAD-Pred) has continued to be, in our series (about 60 cases) and others', the single best available treatment for children and adults (without or with an associated cancer). It has the greatest benefit/side-effect ratio and is easiest to manage. However, because not all patients respond, we are defining some of the parameters of their immunologic response to prednisone and seeking predictive parameters thereof.

By use of T- and B-lymphocyte cell markers, T- and B-lymphocyte mitogens, and T-lymphocyte cytotoxicity on tissue-cultured chromium-labeled muscle fibers, we have now published that in PM/DM patients, while HSDAD-Pred is clinically cumulatively effective for months and longer, its measurable effect on the peripheral circulating lymphocytes, using currently available techniques, lasts less than 24 hrs; and these effects are more profound on the T-lymphocytes. We are now correlating these data with concurrently obtained blood levels of prednisone and prednisolone to determine any hypoabsorption or hypercatabolism of the drug (with P,CC). In patients, children and adults, inadequately responding to prednisone we have found on an occasional-case basis some remarkably therapeutic responses to added azathiaprine (3 mg/kg/d) -- to more clearly establish the efficacy of azathiaprine, a double-blind trial is being conducted. We are also measuring the patients' lymphocyte responses to azathiaprine.

Massive subcutaneous calcification can be a disabling complication of childhood DM. We have found new ways to clinically detect it in its early stages, isotopically with ^{99m}Tc -diphosphonate body scanning (with NM,CC) and by xerography (for microscopic detection methods, v.i.) (with R,CC). However, our therapeutic trials with diphosphonate have not been beneficial (also not beneficial in a patient with myositis ossificans). However, in some severely affected patients the calcium has remarkably diminished as the muscle and skin were responding to our combined azathiaprine-HSDAD-Pred pro-

gram. The reasons for this calcification are being sought by our histochemical, electronmicroscopic, and autoradiographic studies (v.i.). The exact mechanism of muscle damage in PM/DM is unknown. Previously we have reported immunoglobulin complexes deposited in blood vessels in 83% of the childhood cases and 29% of adult cases, findings supporting our earlier hypothesis that an aspect of muscle damage may be vascular. While disorders of both immunoglobulins (produced by B-lymphocytes) and direct immunocyte toxicity (T-lymphocytes) might be occurring in all cases of PM/DM, perhaps the former (deposited as intravascular immune complexes) are more muscle-damaging in the childhood form and latter are more pathogenic in the adult form. We are now attempting to correlate a possible vascular mechanism with our histochemical finding of preferential perifascicular involvement (occurring especially in childhood DM), as well as relating our findings in patients to our experimental animal model of muscle ischemia (v.i.). It is conceivable that the immunologic abnormalities of PM/DM are ultimately provoked by a viral infection; however, our attempts to see or "rescue" a virus from PM/DM patients' muscle directly after tissue culture of it, or after animal inoculation, thus far have been negative (with NYU, ID-NINCDS, and NCI).

A new and possibly disease-characteristic histochemical finding in PM/DM patients of microscopic foci calcium accumulation in collagenous connective tissue of muscle and subcutaneous regions has been demonstrated histochemically at both the light-microscopic (alizarin-red method) and EM (pyroantimonate method) levels. Its pathogenesis is being sought, as is its correlation with our positive ^{99m}Tc -diphosphonate patient-scans for calcium, our autoradiographs of patient skin and muscle biopsies for calcium, and our previously described highly-disease-characteristic histochemical finding of alkaline-phosphatase staining in the intramuscular connective tissue.

Duchenne muscular dystrophy (DMD) is an hereditary progressive deterioration of muscle in children, usually causing wheel-chair or bed confinement by age 12 and death by age 20 years. Cause and treatment are not known. Our studies involve improving diagnostic methods, seeking the basic cause, and trying experimental therapy when available. We also seek fuller understanding of the basic biology and pathologic responses of muscle. The other myopathies and the other neuromuscular diseases of unknown nature which we study are often devastating to children or adults, and in their study our general objectives are the same.

Current competing hypotheses of the pathogenesis of DMD are: (a) primary or secondary defect of blood supply to muscle, (b) primary defect of energy source within the muscle fiber, and (c) primary muscle-fiber membrane defect. Although many others favor c, we favor a or b. Some of the findings in DMD muscle considered by others to be supportive of c we consider to be invalid results or not distinguishing between c and b. The defect of erythrocyte morphology reported by others we are reporting as not present in our controlled studies. The defect of the muscle-fiber membrane evident by peroxidase penetration reported by others we find not disease specific. The alteration of muscle homogenate adenylcyclase reported by others we have reservations about because (i) it wasn't exclusive to DMD, and (ii) its cellular site (presumed by them to be muscle fiber sarcolemma) was not known from those biochemical studies. Our own newly developed histochemical method for adenylcyclase (AC)

shows highest muscle AC in blood vessels and almost none detectable in normal muscle, but fairly high amounts of AC diffusely throughout the interior of regenerating fibers, especially evident in DMD biopsies.

Our new model involving the focal application of a mitochondrial poison to muscle fibers in situ, which results in the secondary loss of sarcolemmal membrane integrity of the damaged fibers, establishes an experimental basis for at least the possibility of hypothesis b, in harmony with our clinical demonstration that in muscle phosphorylase deficiency ischemic exercise precipitates acute breakdown of the sarcolemmal membrane barrier, allowing marked creatine-phosphokinase (CPK) egress and calcium ingress (detected by scanning with ^{99m}Tc -diphosphonate).

Our ischemia hypothesis for DMD, which proposes a functional defect on the arterial side of the vascular tree, has been based on our studies of the histochemopathology of DMD muscle and of our experimental ischemic myopathy in animals. As yet, an ischemia mechanism, although possible in DMD patients, has not been demonstrated in them directly. We are, though, now reporting that in known ischemic human muscle, caused by occlusive vascular disease, the pattern of histochemopathology of muscle (groups of necrotic or regenerating fibers in fields of normality) overlaps that of early DMD and of our experimental ischemic myopathy in animals, thus at least being harmonious with the ischemia hypothesis (with VAH, D.C.). A number of investigators are now attempting to evaluate our ischemia hypothesis. Although some feel they are disproving it, we have in a published formal forum provided counter-arguments of those criticisms suggesting that they are incorrectly based or inconclusive.

We used the ischemia myopathy model to study some of the details of the mechanisms of muscle fiber damage. One result was the development of a new, rapid and simple radioisotopic method, based on uptake of ^{99m}Tc -diphosphonate, of quantitating active skeletal muscle damage in experimental animals -- that uptake was directly correlated with other more arduously determined parameters of muscle damage, loss of muscle K^+ and elevation of plasma CPK (with AFRRI). We have now shown that those parameters are also directly correlated with uptake of ^3H -diphosphonate and with calcium uptake, the latter presumably the basis for the diphosphonate accumulations. Our autoradiography shows the uptake of ^3H -diphosphonate to be within muscle fibers, ones necrotic and even intact ones (presumably pre-necrotic or perhaps reversibly injured) -- this correlates very well (in histochemically-stained serial sections) with calcium accumulation within and complete loss of phosphorylase/glycogen staining from those fibers. We have shown that calcium accumulation in damaged muscle fibers is also evident by light- and electronmicroscopy-histochemistry in various human disorders -- it is not disease-specific but it is a new way to identify damaged fibers, including minimally affected ones not identifiable by other methods. Our electronmicroscopy-histochemical method (pyroantimonate) shows the calcium increase in the pathologic fibers to be in mitochondria > sarcoplasmic reticulum > myofibrils. Nuclei (nucleoli and heterochromatin) are also calcified. These are the same organelles we find calcified when normal intact muscle is soaked in a high calcium solution.

From all of these studies we have formulated a new hypothesis covering molecular aspects of muscle fiber damage, as follows: (a) any damage to the sarcolemma -- be it exogenous (e.g., trauma, ischemia), endogenous deficiency

of energy supply (e.g., phosphorylase deficiency, mitochondrial poison) or, conceivable but not tested, a direct primary or secondary sarcolemmal defect -- can break the normal sarcolemmal barrier to calcium (Ca^{2+} / Ca_i^{2+} normally about 10,000-20,000/1); (b) the influxing Ca^{2+} is taken up by those organelles known to normally have the greatest capacity to do so, i.e., mitochondria > SR > myofibrils, and nuclei; (c) this heavy calcium accumulation eventually can damage organellar function -- e.g., a high Ca^{2+} is known to reduce mitochondrial ATP synthesis, which presumably would lead to further impairment of the sarcolemmal barrier and increasing damage of the muscle fiber; (d) the molecular death of the fiber may be due to the heavy calcium influx; if only mild influx recovery might occur.

Along with these theoretical and experimental data, we have demonstrated that patient scanning with $^{99\text{m}}\text{Tc}$ -diphosphonate can be used to detect abnormal uptake, presumably based on abnormal accumulations of calcium, in various disorders of muscle. There is: (a) greatest uptake in the polymyositis/dermatomyositis complex (probably enhanced by diffuse radiologically-invisible but histochemically and electromicroscopically-evident calcium in connective tissue of muscle and subcutaneous regions), (b) only moderate uptake in DMD even though the patients have very high serum CPK; (c) normal or minimally increased uptake in neurogenic involvement; (d) surprising moderately high uptake, in the presence of normal serum CPK's, in muscle of patients with ragged-red fibers (which contain abnormal mitochondria); (e) very high uptake in phosphorylase-deficiency muscle acutely damaged by ischemic exercise and in muscle acutely damaged by trauma (viz., at the site of muscle biopsy).

A survey chapter (>500 references) of the biology of the muscle cell in relation to myopathies has been written.

Other myopathies. Tissue culture of human muscle biopsies provides growing muscle fibers free of all neural, vascular, and humoral factors present in the patients. After we developed a new program (reported last year) for achieving abundant, reproducible and mature growth of human fibers in culture, including spontaneous twitching, and for precisely selecting certain fibers for enzyme histochemistry, immunohistochemistry, EM, or EM-histochemistry, we turned our attention to culturing the biochemically and the morphologically distinct myopathies.

In adult-onset acid maltase deficiency we have reported the first "reincarnation" in cultured muscle fibers of the biochemical and morphological defects characteristic of the disease -- the muscle fibers in the biopsy and the ones newly grown in culture (with NYU) were identical by histology (vacuoles), histochemistry (acid phosphatase high in the vacuoles), electronmicroscopy (glycogen accumulated in lysosomes) and biochemistry (with Columbia) (absent acid maltase by the natural substrate method, 5-10% of normal acid maltase by the artificial substrate method, normal neutral maltase), normal kinetics of neutral and the trace-amount acid maltase, elevated acid phosphatase. This is the first establishment of a neuromuscular disease as a primary myopathy. It provides a new test system for manipulations directed toward the treatment or prevention of muscle-fiber damage in this disease without inducing any risk to the patient. Further, we have now similarly shown reincarnation of the severe biochemical and morphologic defects in 4 chronic-infantile cases of acid maltase deficiency and partial defects in 3 heterozygotes.

We recently reincarnated a second metabolically distinct myopathy, phosphofructokinase (PFK) deficiency. Extremely reduced levels of that enzyme were found in muscle fibers cultured from a patient with a new combined syndrome of muscle and erythrocyte PFK deficiency and hypolipoproteinemia (with NYU and Columbia). Growth of that muscle in culture was enhanced by B-hydroxybutyrate added to the medium, a ketone-body providing one of the alternate energy sources which that muscle must be depending on since its glucose-utilization pathway is defective (with NYU).

In a third glycogenolytic-enzyme defect of muscle, phosphorylase deficiency, we have confirmed our previous finding that the enzymatic absence of the muscle fibers is "cured" with their growth in culture -- actually the remarkable and unexpected recovery of phosphorylase activity is seen in fibers in the regenerative state in culture and *in vivo* (with NYU). The therapeutic thrust need now is to provoke appearance of that phosphorylase in the mature fibers of the patient.

A morphologically characteristic defect, "cabbage bodies" (multilaminated material in lysosomes) in muscle fibers of a patient with a chronic myopathy, were reproduced in his cultured muscle fibers (with NYU). Although the biochemical defect is not known, both the biopsied and cultured muscle had elevated acid phosphatase, suggesting the patient has a defect of a yet-unpinpointed lysosomal hydrolytic enzyme (with NYU and Hopital des Enfants Malades, Paris). We are assaying 12 different lysosomal enzymes in our cultures of human and animal muscle to search for defects thereof (with NYU and Hopital des Enfants Malades, Paris).

"Raggéd-red" muscle fibers, which contain severe mitochondrial abnormalities, are the commonest histochemical accompaniment in limb muscles of the mixed syndrome of progressive external ophthalmoplegia in our series of 41 patients (after myasthenia gravis and myotonic atrophy are excluded). Some, though not all, of those mitochondrial changes have been reincarnated in limb muscle cultured from such patients (with NYU) -- the mitochondrial crystal-like inclusions have not been. In the original biopsies those mitochondrial inclusions usually lacked cytochrome oxidase staining by our EM-cytochemistry.

In tissue cultures of "normal" chick embryo skeletal muscle we were able to induce certain bizarre changes of mitochondrial morphology by pulses of dinitrophenol (DNP), but did not produce all the mitochondrial changes occurring in the human "ragged-red" fibers (with NYU). In the course of that study we found that DNP markedly promoted the detectability of avian oncornavirus (the now-preferred name for the group which includes leucosis and Rous sarcoma viruses) in all such "normal" chick muscle cultures -- virus was evident morphologically (C-particles) and by complement fixation titers (COFAL) (with NYU). This demonstrated that the "normal" chick embryo muscle cultures so frequently used by various investigators for physiologic, biochemical, immunologic and developmental studies are actually virally-contaminated test objects. The question of whether that oncornavirus has a "normal" role in development of "normal" chick muscle *in vivo* or *in vitro* is raised by this study. Our finding the C-particles exclusively in dilatations attributed to T-tubules raises the intriguing possibility that T-tubules might be the aqueducts of virus infestation of or shedding from muscle fibers. The question

of viruses harbored in mitochondria (as "mitochondriophages", analogous to bacteriophages) is raised by the C-particles being provoked by DNP, an uncoupler of mitochondria oxidative phosphorylation. Finally, we have raised the speculation that such mitochondriophages might be responsible for the changes in the ragged-red-fiber diseases.

A new type of mitochondrial abnormality, light-cored dense particles, has been reported in skeletal muscle fibers of a patient with cardiac "asymmetric septal hypertrophy". A new neuromuscular disorder, striped loss of mitochondria, has been found in a family with dominantly inherited distal muscle weakness. In biopsies of 3 patients with sporadic chronic myopathies we have by electronmicroscopy found under the plasmalemma long strands suggesting viral material. In a patient with late-onset rod disease, an entity first described by us a few years ago, an associated unusual serum paraprotein, IgG/lambda myeloma protein has been found. Its significance is not known.

We have now reported in 3 cases a new clinically-delineated "levitated arms syndrome" and identified the cause as muscle fibrosis resulting from iatrogenic intramuscular injections into the deltoids, especially of pentazocine (Talwin). One patient also had levitated legs (evident when sitting) from pentazocine injections into the rectus femoris muscles. Two were successfully treated by surgical transection of fibrous bands, in the deltoids of both and also the recti femoris in one. These cases demonstrate a newly-recognized, preventable, and treatable iatrogenic disease.

Neuromuscular disorders of uncertain classification. Selective atrophy of the type-II (glycolytic-rich, oxidative poor) muscle fibers, especially the subtype IIB fibers, has been shown to be the basis of cachectic atrophy accompanying cancer and other chronic debilitating disorders. We have now reported a detailed analysis of this phenomenon and formulated possible hypothetical mechanisms. They are: (a) a "Sparafucile" factor (v.i.), directly or indirectly resulting from the cancer, indiscriminately assassinating the type-II fibers, vs. (b) a muscle-fiber martyrdom mechanism of protein catabolism to supply energy substrate, via the alanine shunt and glyconeogenesis, to cells more vital to the organism. The possible neurogenic vs. myogenic pathogeneses of the type-II atrophy have been analysed and theoretical models based on empiric findings constructed. Evaluation of the mechanism of type-II fiber atrophy in cancer patients is important because this "remote effect" muscle weakness is often the most crippling aspect of cancer -- if the molecular mechanism can be discovered it might be treatable independently of treatment and response of the cancer itself. And improvement of the muscle weakness and wasting could even make the patient better able to withstand the rigors of direct anti-cancer therapy.

Malignant hyperthermia (MH) is a syndrome, 70% fatal, of acute rise of body temperature and muscle rigidity during general anaesthesia. A number of the patients have underlying not-well-defined neuromuscular disorders. In one MH patient, and his father, we have identified central-core disease (CCD), with its typical type-I muscle fiber predominance (with Children's Hospital, Wash., D.C.). Because there are in the literature two other families with CCD and MH, we have issued a caution to all our CCD patients

and their physicians regarding the possibility of MH during general anaesthesia. The mechanism(s) of the attack of MH is not known. It appears that there is an excess of free intracellular calcium in the muscle fiber, which we propose might be due to an effect of the anaesthetic or muscle-relaxant agent on the calcium-barrier function of the sarcolemma (v.s.). We are now investigating why central core disease, which we have earlier postulated to be due to a congenital monophasic neuropathy mainly affecting the type-II units, should predispose to the development of MH.

Myotonic atrophy (myotonic dystrophy) is of unknown pathogenesis. We have previously raised the possibility of at least partially a neurogenic aspect. To evaluate myotonic phenomena by various parameters we have set up the animal model of 20,25 diazacholesterol-induced myotonia (originally described by others). Although the animals become markedly myotonic, we find that their muscle reveals no histochemical alterations.

The systemic approach in application of a rapid short (5-stain) and long (18-stain) battery of histochemical reactions to fresh-frozen sections of human muscle biopsies (including basic stains developed, e.g., our modified trichrome) and the concepts in the analytical approach to neuromuscular pathology we have developed are being followed in nearly all centers for neuromuscular research throughout the world.

Cross-category and basic aspects. Previously we have formalized and seen adopted by the World Federation of Neurology our long-used nomenclature for histochemical types of human limb-muscle fibers, based on two types, I and II. One of our former trainees has shown histochemical subtypes of the type-II fibers. Now we have published histochemical distinction of two subtypes, IA and IB, of the type-I fibers and shown selective involvement of a subtype in certain human neuromuscular disorders (with NYU).

Eye muscle fibers histochemically are not exactly like limb muscle fibers. We have demonstrated their normal histochemical patterns in Rhesus monkey and identified 3 types, "fine", "granular" and "coarse". The first two types have one endplate per fiber and probably are types of twitch fibers; the last has multiple endplates and may be a tonic fiber. Following denervation the first two developed extrajunctional acetylcholine receptors but the coarse fibers did not -- no fibers were positive beyond 13 weeks post-denervation.

The autoradiography laboratory was established in our own Branch this year. We have begun with ^{32}P -diphosphonate to demonstrate intracellular calcium (v.s.). Now we are developing a technique for doing autoradiography of biopsy samples following injection of some of the short-lived gamma-emitting radionuclides used in patient scanning, to establish direct scanning-histoautoradiographic correlations.

Mammalian sarcolemmal membranes have received major biochemical emphasis. Results of the following studies are now being published. Not only have pure fractions of rat sarcolemma been obtained, but methods have been perfected that provide adequate quantities of sarcolemmal membrane from human muscle obtained from limb amputation or radical mastectomy. With the sarcolemmal membrane fractions and subfractions, methods have been established for studying

acetylcholine receptor, acetylcholinesterase, $\text{Na}^+\text{-K}^+$ ATPase (Na^+ -stimulated phosphorylation), adenylylase, divalent cation (viz., Ca^{++}) binding/transport, and Ca^{++} -stimulated ATPase. Some of these have been studied in sarcoplasmic reticulum fractions as well. Elucidated have been detailed properties of: (a) the adenylylase (i.e., fraction localization, kinetics, catecholamine-activation, guanylyl-inidodiphosphate activation, insulin and glucagon inhibition, and response to denervation in "red" cf. "white" muscle of animals); (b) the sarcolemmal protein phosphorylation (i.e., Na^+ enhancement blocked by K^+ , phosphorprotein state suggesting an acylphosphate bond, high turnover rate suggesting it is a functional intermediate of Na^+K^+ ATPase, and molecular weight); (c) the effect of denervation on this transport ATPase system; (d) the Ca^{++} uptake and release by human sarcoplasmic reticulum (SR) (i.e., by use of specific antibodies made against SR which block the Ca^{++} uptake and inhibit adenylylase but do not affect ATPase activity, suggesting different localization of these functions within the SR); (e) the role of bound-calcium in the regulation of Ca^{++} transport by SR; and (f) physicochemical properties of ACh-receptor cf. acetylcholinesterase. These assays are now being used to seek biochemical defects of sarcolemmal or sarcoplasmic reticulum function in muscle biopsies from patients with various neuromuscular disorders.

Insulin receptor function is being analysed in our cultured chick muscle fibers as the first step toward studies of diabetic neuropathy patients (with Georgetown U.). The effect of dibutyryl cyclic AMP and a cAMP-phosphodiesterase inhibitor (phthalazinol) on growth and maturation of human and animal cultured muscle fibers is being evaluated (with NYU).

Periodic paralyses (PP) are hereditary or acquired disorders causing chronic weakness punctuated by attacks of paralysis. Associated metabolic abnormalities are known but the actual pathogenic mechanisms are not. Standard palliative preventive therapy in the idiopathic hypokalemic form of PP is potassium, and more recently acetazolamide. Our studies involve improving diagnostic methods, seeking the causes and analysing the pathogenic mechanisms, and improving methods of treatment.

In the hypokalemia form of PP, the treatment we introduced, long-term acetazolamide, has continued to be the best prophylactic agent both for preventing attacks and improving inter-attack weakness. It is now in the textbooks as such. Two of our patients have been treated successfully for more than 10 years. Renal calculi in one of 26 patients, possibly but not definitely related to the acetazolamide, have been the only suspected side-effect. Since muscle does not contain carbonic anhydrase, the mechanism of acetazolamide benefit in hypokalemic PP remains unknown -- we are investigating it.

Myasthenia gravis (MG) is an acquired disorder affecting transmission at the neuromuscular junction, mainly in adults and older children. The primary cause is not known but the pathogenic mechanism is considered to be "autoimmune" or "dysimmune". Untreated patients are usually seriously handicapped and many die. Palliative treatment with anticholinesterases and anti-pathogenic treatment consisting of thymectomy, ACTH and, most recently, prednisone has helped considerably but much disability, some fatality, and drug side-effects persist. Our studies involve improving diagnostic methods, seeking the cause and analyzing the pathogenic mechanisms, and improving methods of treatment. We also seek fuller understanding of the basic biology and pathologic responses of the neuromuscular junction.

Last year we reported the identification of a newly recognized blocking factor, an IgG, in the sera of MG patients which blocks binding of alpha-bungarotoxin (α BT) to the human junctional acetylcholine receptor (AChR) at the normal neuromuscular junction (41% of MG patients) and extrajunctional AChR of denervated human fibers (72% of MG patients, including all who had a thymoma) -- the latter being the more sensitive assay -- presumably because the factor itself is binding to the AChR (with NIHL). Now we have reported that in a correlative study all MG patients having the IgG antimuscle antibody (first found by others) also had the blocking factor (although only half having the latter had the former) (the only discordant finding was in one non-myasthenic myositis patient with thymoma who had antimuscle antibody but no detectable blocking factor) -- we suggest these may be the same antibody or, if different, apparently virtually always co-produced. The junctional localization of the blocking factor puts it in the correct position to impair neuromuscular transmission and cause the weakness of MG. Although we think this is likely, the factor has not been shown to produce weakness in any test system. We have not been able to induce weakness of newborn mice by injecting IgG blocking-factor-positive MG sera during pregnancy or into the newborns. Blocking-factor-positive MG sera were shown to block the binding of α BT to the diffuse extrajunctional receptors of aneurally cultured human, rat, and chick muscle fibers (with NYU).

Since we have shown by electromyographic-immunohistochemistry that α BT binds, and presumably localizes AChR, at the neuromuscular junction both to the crests of the post-synaptic muscle membrane folds and to a lesser extent to the pre-synaptic axonal membrane, the blocking factor, if indeed pathogenic, would act both post- and pre-synaptically. Data from other types of studies by others support a pre- as well as a post-synaptic locus of AChR in normal neuromuscular junctions. On the basis of the blocking factor, we have revised our multistep hypothesis of the pathogenesis of MG and in doing so realized its striking analogy to the scenario of Verdi's *Rigoletto*. We therefore term the presumably-detrimental blocking factor a "Sparafucile" molecule after Verdi's hired assassin who without malice killed the unintended victim, in fact, killed the beloved daughter, Gilda (the AChR receptor in MG) of *Rigoletto*, who planned the assassination to be of the Duke of Mantua. It is evident from further analysis of the scenario that the answer to MG will only come with the identification and treatment or prevention of Count Monterone's curse (? an environmental factor, ?? viral).

We are investigating which cells, presumably B-lymphocytes, make the blocking factor in MG patients, and why, and how its production or presumed detrimental action can be prevented.

That the thymus is involved in many cases of MG is well known, but its role is not. Our counts of T- and B-lymphocytes in fresh thymus removed from MG patients during therapeutic thymectomy have not confirmed an increased percentage of the latter as reported by others. Mixed lymphocyte reactions with MG thymus are being analysed by light- and electronmicroscopy. Our EM of MG thymuses has thus far failed to reveal a virus, as have tissue culture studies. The multifactorial nature of the dysimmune phenomena in MG was reemphasized by the finding of anti-native-DNA antibody in the sera of a significant number of patients (with NIAMDD).

Working with the induced autoimmune model (rabbits injected with electric-fish AChR), originated by others, of experimental allergic MG (EAMG) (with Cornell) we demonstrated: (a) binding of that rabbit sera to human neuromuscular junctions but not to extrajunctional receptor at light-microscopic resolutions, (b) binding of it by electronmicroscopic resolution to the plasmalemma of cultured human, rat and chick fibers (with NYU), (c) binding of that sera to the original antigen in a radioimmunoassay we developed (with IB), (d) similarities but also distinct differences of the model with human MG, indicating it is not a perfect model of the latter although it could still be a model-in-principle, (e) toxicity of EAMG sera to reinnervated muscle in tissue culture which did not exceed the toxicity of normal rabbit sera (surprisingly toxic).

Confirmed and adopted by most other physicians has been the treatment we introduced to MG, long-term high-single-dose alternate-day prednisone (LT-HSDAD-Pred). In our own series it continues to be extremely beneficial in the majority of cases, 37 of 40, and for as long as 10 years in a child and 6 years in an adult. Responding best are the older-onset patients, especially the older males. Our 3 non-responders were females in the menstruating age group. Interestingly, none of our responding patients has become absolved of his requirement for prednisone, even after a gradual tapering of the dose. For example, two patients have exacerbated 3 months after stopping a 5 mg q.o.d. dose (this is about the length of time taken for resumption of abnormal IgG synthesis as measured by others in another disease). We have recently modified the treatment slightly by giving, to patients not simultaneously taking anticholinesterase drugs, the single-dose 100 mg prednisone daily for the initial 2-4 weeks before converting to the alternate-day schedule, apparently resulting in more rapid improvement. Fragile patients are not taken off their anticholinesterases but in them prednisone is given in a gradually incrementing single-dose-daily schedule beginning with 10-20 mg. Regarding combining anticholinesterase and prednisone, we find that low doses of one can be combined with the other advantageously -- but that patients taking both drugs sometimes seem to have a more "brittle" myasthenia and must be watched carefully for intractable overdosage by the anticholinesterase. However, because neither anticholinesterase nor prednisone treatment is either curative or completely preventative, more details of the pathokinesis are needed (v.s.).

The effect of the HSDAD-prednisone treatment on lymphocytes was measured over a 48-hour cycle in a number of MG and other patients. At 6 hrs. after the 8:00 a.m. prednisone dose there is marked depression of T-lymphocyte counts and lymphocyte responses to T-lymphocyte mitogens with a lesser effect on B-lymphocytes and response to B-lymphocyte mitogens, and there was return of these measurable effects to normal by 24 hrs. after the dose -- yet clinically the prednisone has a cumulative effect over weeks and months.

Partial sternal-splitting continues to be our preferred approach to thymectomy (with NIHL) -- the mortality is essentially zero. The suprasternal approach for us is inadequate for satisfactory removal of all thymic tissue. We continue to demonstrate that "out-of-control" MG patients often can be remarkably improved by treating co-existent medical problems such as chronic respiratory infections, urinary tract infections, and anemias -- possibly co-existing, ameliorative problems must be sought in each MG patient by detailed general medical investigation.

The remarkable ancillary benefit that broad-aspect nursing can provide to an MG patient is repeatedly evidenced in our patients -- our multidimensional nursing care approach for myasthenics has been videotaped (with Nursing, CC) and made available for general distribution -- this undoubtedly will help improve the care and perhaps save the lives of some myasthenic patients with serious disease, especially in hospitals not frequently caring for such patients.

With i.v. edrophonium (Tensilon), contrary to its usually considered short action of 5-10 min., we have preliminarily reported the documentation by detailed clinical and electromyographic testing an improvement in strength and neuromuscular transmission lasting 1-2 hrs. in several MG patients. This has practical importance since repeated edrophonium tests are commonly used (overused), without careful vital capacity monitoring, for adjusting dosage of other anticholinesterases.

A new electrophysiologic type of defect of neuromuscular transmission has been found in a patient with a fatigue syndrome and chronic renal disease -- after a brief tetanus there is a diminished but broadened total-muscle action-potential which gradually recovers during rest or slow stimulation, and it is not ameliorated by edrophonium, guanidine, or Ca^{++} .

We have just completed setting up the electromyographic equipment to study "jitter" and "blocking" phenomena recently described by others -- it is important diagnostically and investigatively in MG patients.

Amyotrophic lateral sclerosis (ALS) is a progressive neurologic disease affecting motor neurons, of adults, usually leading to death within 1-3 years of onset. The cause and treatment of ALS are unknown. Our studies involve improving diagnostic methods, delineating subtypes of ALS and other possibly treatable diseases mimicing ALS (several cases of which we have found in the past year), seeking the basic cause and trying experimental therapy when available. We also seek fuller understanding of the basic biology and pathologic responses of the motor neurons. We have especially pursued a possible metabolic cause, but have also looked for evidence of viral causation.

Detailed, repeated and reproducible radioimmunoassay studies of more than 125 patients have revealed that in ALS patients there is a distinctly low cerebrospinal fluid (CSF) level (compared to paranormals and myopathy and polyneuropathy disease-controls) of cAMP, and a slight decrease of cGMP (with Naval Medical Center). I.V.-probenecid studies demonstrate this is due not to increased clearance of cAMP or cGMP from CSF but probably to decreased synthesis in the neural tissue (or perhaps increased breakdown, or both). The low levels of cAMP, but not of cGMP, were corrected with a phosphodiesterase inhibitor, phthalazinol, which had no effect on plasma cAMP and cGMP (with Institute of Japan Atherosclerosis Research Foundation). The significance of this newly recognized biochemical defect in the pathogenesis of ALS remains unknown. We have shown, in ALS and non-ALS patients, that cAMP in plasma does not cross the blood-brain barrier during acute elevations -- glucagon raised plasma cAMP 40-fold and urine cAMP rose, but there was no change of cAMP in CSF; no change in cGMP of plasma or CSF followed glucagon. Thus, CSF levels of cAMP reflect only metabolism of it within the central nervous system. The glucagon caused a 2-fold rise of blood glucose and a concomitant rise in CSF glucose. CSF cAMP and cGMP are somewhat elevated in patients with spasticity of various causes (excluding ALS).

The cells from which cAMP in the CSF arises are not known. We have developed a new technique for histochemically demonstrating adenyl cyclase, the enzyme synthesizing cAMP. It shows, in the spinal cord, cerebellum, brain stem and cerebrum, that the greatest amount is in the blood vessels with much lesser amounts in neural or glial tissue; almost none occurs in purkinje cells, while anterior horn motor neurons have slight to moderate amounts. A large amount was also in the epithelial cells of the choroid plexus and in the pineal cells. In lower motor neurons (LMNs) cAMP could, if its presence there is significant, conceivably have some role in excitatory or inhibitory transmission, in proximately or remotely generated trophic effects, and/or in internal metabolism such as in the rich glycogen metabolism machinery (that we have previously demonstrated to be a special characteristic of LMNs and cortical motor neurons and which, therefore, could conceivably be the site of the defect causing ALS).

Previously we have reported a neurologic abnormality with certain features like ALS in many cases of primary and secondary hyperparathyroidism; we have also studied a few cases with both frank hyperparathyroidism and frank ALS. To study the possible role of abnormality of parathyroid function and/or calcium metabolism in ordinary ALS, we have done radioisotopic calcium retention tests (n=80) on ALS patients and disease-controls: we have found abnormally low calcium retention in 63% of ALS, 60% of polyneuropathy, and

only 35% of myopathy patients (with NNM). So far, treatment with 20,25 dihydrotachysterol has been found to reverse the calcium-retention defect in some ALS patients but not result in clinical improvement.

Phthalazinol treatment of ALS patients, although able to raise their low CSF cAMP, has not resulted in consistent therapeutic benefit to date.

In ALS patients, including those with a late-post-polio progressive muscular atrophy syndrome, we continue to search for evidence of a viral cause in ALS sera, CSF and tissues by viral antibody profiling (polio 1,2,3, mumps, measles, rubella, coxsackie, and influenza A and B (with ID, NINCDS)), and various tissue culture techniques (including activation techniques and evaluation by morphologic, antigenic, and reverse transcriptase assays). To date no viral evidence has been found. Nevertheless, an animal model of reactivation of a latent virus infection was established -- latent infection of mouse dorsal root ganglia with herpes simplex virus type 2 or 1 could be reactivated by injuring the peripheral nerve (with NIDR). Evaluation of HLA types has not revealed any predominant type represented in our ALS patient group.

From our cumulative experience with more than 500 patients with ALS or ALS-like syndromes we emphasize that (a) about 10 are chronic, surviving more than 5-15 years (and therefore giving false hope to experimental therapists if they are not aware of this subgroup), and (b) as many as 15% or so of the cases referred to us as ALS have a different disease, more benign, and occasionally treatable -- they are identified by careful clinical and laboratory examinations.

Two extensive chapters have been written, one on numerous aspects of the biology of the lower motor neurons as a basis for understanding and investigating diseases thereof (>1000 references), and the other on various aspects of the several motor neuron diseases (>300 references).

Previously we have introduced the new concept, on the basis of our combined electromyography (EMG) and histochemistry studies, our open-biopsy-EMG technique, and on theoretical grounds, that an EMG pattern on voluntary effort of brief-duration small-amplitude overly-abundant motor-unit action potentials (BSAPs), sometimes polyphasic (BSAPPs) is just as possibly a reflection of neuropathic as of myopathic diseases -- and we criticized the established (and we would say vacuous cliché of "myopathic EMG" for that pattern. Initially not met with enthusiasm by hard-core electromyographers, our hypothesis is now becoming accepted by at least some of them, as well as by many less tradition bound investigators.

Altered influence of the lower motor neuron on muscle fibers was identified and studied by use of the new α -bungarotoxin immunoperoxidase technique at light-microscopic and EM levels for identifying acetylcholine receptors (AChRs). Especially useful was the visualization of the AChRs when present diffusely in the plasmalemma following denervation. Last year we demonstrated that the diffuse extrajunctional AChR can be used for identifying and "dating" denervated fibers. This year we have used it to look for evidence of altered neural influence in several diseases not generally con-

sidered neurogenic but ones in which we have previously postulated possibly to be on a neurogenic rather than a myopathic basis -- they include central core disease, type-I-fiber hypotrophy with and without central nuclei, myotonic atrophy, myotonia congenita, some cases of benign congenital hypotonia, some of type-II-fiber atrophy, and congenital and adult-onset rod diseases. None of these disorders showed extrajunctional AChRs by light-microscopy -- however, the question is still not fully resolved because: (a) there could be slight extrajunctional α BT binding that is demonstrable only by electron-microscopy, or (b) there could be in some of those diseases a subtle incomplete neurogenic alteration of neural influence partially affecting muscle fiber function and morphology without allowing extrajunctional AChR to develop in those fibers.

Conversely, a new concept of "myogenous mal-innervation" was formulated. It was based on our demonstration that: (a) diffuse extrajunctional ACh-receptors were present on "regenerating-degenerating" muscle fibers in myopathic biopsies of our patients, (b) human, rat, and chick embryo skeletal muscle grown in tissue culture without innervation (pre-innervated muscle) has diffuse extrajunctional ACh-receptors (with NYU), and (c) in adult animals, segments of muscle fibers experimentally separated from their motor innervation point but remaining viable develop diffuse extrajunctional ACh-receptors. Thus we have formulated a "law of mal-innervation", since it becomes evident that the presence of diffuse extrajunctional AChR in muscle fiber plasmalemma is certainly a sign of mal-innervation of the muscle fiber but it is not pathogenesis-specific since it can be either a neurogenous or a myogenous de-innervation or dys-innervation, or a primary non-innervation.

The neurogenously denervated fibers in ALS-patient biopsies with demonstrable extrajunctional receptors were used as the more sensitive assay for finding the blocking factor in sera of myasthenia gravis patients (v.s.).

In patients with scoliosis, our studies have continued to show a wide variety of neuromuscular diseases (by muscle biopsy histochemistry and electromyography) to be associated with and probably causing scoliosis. The most common pathology is some form of neurogenic muscular atrophy. Some of our scoliosis cases were previously considered "idiopathic". We therefore are embarking on a broad collaborative survey to define any underlying neuromuscular pathology in all available cases of "idiopathic scoliosis".

We have utilized new techniques for identifying T- and B-lymphocytes in human cerebrospinal fluid in more than 100 patients. The mean percents of total CSF lymphocytes are, respectively, 72 and 16 (12 being null cells). Changes in various diseases are being investigated, as are the responses to prednisone.

Polyneuropathy (Peripheral Neuropathy) (PN): The peripheral neuropathies comprise a group of disorders of various causes, more than half unknown. They always cause serious physical handicap sooner or later in the course of the disease, sometimes associated with intractable pain and ulceration and loss of feet and hands. Our studies seek to delineate the underlying causes and where possible develop a treatment. We also seek fuller understanding of the basic biology and pathologic responses of the lower motor and sensory neurons and peripheral nerves.

The majority of patients we see are of undiscernable cause. Those which are non-familial we may treat with LT-HSDAD-prednisone, especially if less than 5-years duration. We have had good to outstanding success in more than 25 such patients, most having been given-up on by others and some having come with diagnoses of non-treatable diseases (e.g., ALS or "Charcot-Marie-Tooth" disease). Long-term treatment is required -- too-rapid reduction of dosage too soon often results in exacerbation of disease. Excellent results have been sustained for as long as 11 years in an adult and 8 1/2 years in a child (who at age 20 is still regaining motor skills). Our correlative studies indicate that patients most likely to respond are dysschwannian in type (slow nerve-conduction times), relapsing, with elevated CSF protein, but even some non-relapsing patients without slowed nerve conduction times and with normal CSF have responded to LT-HSDAD-pred.

An often undiagnosed cause of sensory-greater-than-motor neuropathy beginning in adulthood is "idiopathic" amyloidosis. We propose the combination of crystal violet stain with fresh-frozen sections of a muscle biopsy (proven by us by a decade of use) as the choice method of diagnosing this disorder. In our most recent 10 cases of non-familial amyloid polyneuropathy, the onset was in later adulthood (mean age 54); 8 were male. We emphasize that this disorder is associated with, and probably the result of, a plasma cell dyscrasia, detectable in 8/10 of our patients as multiple myeloma, and/or serum and/or urine "paraprotein" immunoglobulin fragments. We propose that the neuropathy is due to a systemic metabolic abnormality, possibly related to a circulating abnormal protein fragment (i.e., a Sparafucile phenomenon, v.s.), rather than to pressures from multifocal "amyloid" deposits of immunoglobulin fragments. Our treating 6 amyloid patients with melphalan, an "anti-myeloma" agent, has not been of obvious value. A radioisotope scanning method has proved to be a new diagnostic technique for identifying amyloid in soft tissues -- highly suggestive of this diagnosis is (in the presence of a normal soft-tissue x-ray) a diffusely positive scan, presumably based on binding of the tracer to cations that are binding to the very anionic amyloid fibrils, a mechanism supported by our EM histochemistry studies of amyloid.

We have recently established combined studies of patient nerve biopsies in vitro -- including in vitro nerve conduction velocities of fast and slow fibers, teased fiber histochemistry, electronmicroscopy, and EM-histochemistry (with autoradiography to be added imminently), allowing more precise and direct multidimensional analyses of the afflicted nerves in polyneuropathy patients.

Anterograde axoplasmic transport of protein, glycoprotein and other substances from the neuron soma to the periphery is critical in maintaining integrity of the peripheral nerves. There is also a retrograde axonal transport, the normal role of which is uncertain but, we hypothesize, may serve for: (a) continuous monitoring by the soma of its distal self (e.g., regarding the necessity or not for axonal regeneration), (b) monitoring the external environment of its tip, and (c) acquisition of nutrients; it may also serve as the mechanism by which toxic substances are acquired (v.i.). Our autoradiographic studies (with Johns Hopkins) have demonstrated in animals a rapid anterograde transportation to the axon synaptic terminal at the neuromuscular junction and accumulation there of large amounts of protein and glycoprotein synthesized in the lower motor neuron soma from precursors leucine and fucose injected into the ventral horn less than 24 hrs. earlier. This demonstrated that these rapidly transported axonal proteins and glycoproteins go rapidly to the geographic location where they can be used, hypothetically, for (a) remodeling of the axonal tips and (b) trophic influence on muscle -- both possibilities we are studying further. Combined electronmicroscopy and autoradiography demonstrated that ligation of the nerve interrupted both anterograde and retrograde rapid axonal transport, causing intra-axonal accumulation of masses of smooth vesicular membranes on both proximal and distal sides of the ligation and of substances labeled from precursors injected centrally or peripherally respectively. Those results suggest that fast anterograde and retrograde axonal transport are very similar processes carrying predominantly membranous organelles and constituting a system of bidirectional fast transport (which is interrupted focally by the nerve ligation). Studies of axonal transport provide a means for investigating the origin and fate of axonal organelles in pathologic processes. Our autoradiographic studies have also shown: (a) direct evidence for retrograde intra-axonal transport of tetanus toxin, and (b) that fast anterograde axonal transport provides a necessary contribution to motor nerve regeneration in experimentally sectioned nerves.

A new principle/model for inducing an experimental allergic neuropathy (EAN) in animals has been demonstrated. It involves immunization with soluble nerve protein (in contrast to lipid-associated protein of myelin used in previous EAN models). This represents a new potential model of some human dysimmune dysneuronal peripheral neuropathies, such as in some patients we have seen with prednisone-responsive neuropathies without demonstrable schwann-cell involvement. It also represents a new approach to studying certain dysimmune disorders of the CNS, such as multiple sclerosis and parainfectious encephalopathies. Since our EAN animals also have a component of blockade of neuromuscular transmission that is responsive to edrophonium, the model may have some relevance to myasthenia gravis or other disorders of the neuromuscular junction.

The group of spinocerebellar degenerations comprises diseases of various causes, a few known, most not. They always result in serious physical handicap sooner or later in the course of the disease, and sometimes mental deterioration. Our studies seek to delineate the underlying causes, and where possible attempt to develop a treatment. Previously, we have delineated some specific disorders within this group, e.g., acanthocytosis-with-normal lipoproteins and pyruvate decarboxylase deficiency (ataxia

intermittent), and defective oxidation of puruvate in some patients with the Friedreich's ataxia sub-syndrome. Now we have identified a patient with spinocerebellar degeneration as having "sea-blue histiocytosis". In one of our familial cases of slowly progressive dementing spinocerebellar degeneration syndrome fatal at age 17, we discovered intraneuronal storage material, some lipofuscin-like and PAS-positive and some PAS-negative. This material has now been identified by others as G_{M2} -ganglioside.

cAMP is thought by some to be a mediator of synaptic transmission of some systems in the cerebellum. Our newly developed histochemical technique for demonstrating adenyl cyclase (AC), its synthesizing enzyme, shows the greatest amount to be in cerebellar blood vessels, especially those adjacent to the bases of the purkinje cells, while the purkinje cells themselves are nearly negative. From this study a concern is raised regarding the possible influence of blood-vessel AC and cAMP in biochemical assays of homogenates of microdissected tissue samples.

Some patients with spinocerebellar degeneration have slow eye movements. It was found that they made abnormally slow refixational eye movements by the saccadic system (i.e., they were slow saccades) rather than by the voluntary pursuit system. This has led to the proposal of a new conceptual scheme of how both normal and defective saccadic eye movements might be generated. Study of a group of related patients with familial late-onset cerebellar ataxia has revealed new information about non-visual control of eye position, since the striking abnormality was a defective smooth-pursuit and fixation system. The patients showed evidence of various non-visual mechanisms of maintaining eye position that have not been previously delineated. Rebound nystagmus was shown to occur in normal individuals if fixation is eliminated -- however it becomes clinically apparent in patients with spinocerebellar degeneration because of a coexisting defect of visually-mediated fixation mechanisms. Thus rebound nystagmus can be interpreted as a manifestation of one of the brain's compensatory mechanisms for maintaining eye position when visual systems are ineffective.

Progressive spastic paraplegia is a progressively crippling disorder of children and adults. The causes are not known. Identified were three unrelated patients with a syndrome of chronic adrenal insufficiency from infancy and juvenile-onset of progressive spastic paraplegia and "onion-bulb" peripheral neuropathy, with normal intelligence (with NIAMDD). A single metabolic defect is postulated to underlie the abnormalities in the neural and adrenal tissues (? an adrenoleucodystrophy variant).

Ophthalm-neurology: We have shown that the various neuromuscular disorders affecting the eyes, if correctly evidenced by their limb-muscle pathology, can be on a neuropathic or myopathic basis. They cause various degrees of handicaps. Our studies seek to delineate the underlying disorder, analyze the neuro-ophthalmologic defect, and, if possible, seek methods of treatment. We also seek fuller understanding of the basic biology and pathologic responses of the eye neuromuscular apparatus. The commonest associated limb-muscle pathology of the progressive external ophthalmoplegia syndrome (in our series of 41 patients, after myasthenia gravis and myotonic atrophy are excluded) is a syndrome characterized by "ragged-red" muscle

fibers in limb muscles, whether or not the limbs themselves are weak. Those ragged-red fibers contain mitochondrial abnormalities of various types. We have partly but not completely re-incarnated those mitochondrial changes in muscle tissue-cultured from such patients; and we have produced mitochondrial changes in cultured normal chick muscle by dinitrophenol (v.s.) (with NYU). Other limb muscle pathology associated with progressive external ophthalmoplegia in our cases includes (i) a vacuolar myopathy plus neuropathy, (ii) type-I-fiber hypotrophy with central nuclei, (iii) only denervation, (iv) only morphologically nonspecific myopathy, and (v) type-II-fiber smallness. The histochemical fiber-typing of normal animal eye muscles and the different post-denervation response of extrajunctional receptor distribution of those fiber-types was shown (v.s.). Abnormalities of eye movement in the spino-cerebellar ataxias and in myasthenia gravis are discussed above. EM-histochemistry for calcium (with NEI) has shown its discrete localization within the sacs of the outer segment of the rod cells of the retina. This suggests that the sacs may have a function of calcium storage-and-discharge with photoexcitation analogous to that of the sarcoplasmic reticulum of muscle. The finding has importance in the hypotheses of photo-electrical coupling mechanisms.

Progressive blindness was described in two children with acute lymphocytic leukemia intensively treated with chemotherapy and radiation and considered a possible complication of that therapy (with NEI).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE OFFICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 00915-15 MN	
PERIOD COVERED July 1, 1975 through June 30, 1976					
TITLE OF PROJECT (80 characters or less) Histochemistry Applied to the Study of Human Neurologic Disease					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: W. King Engel, M.D., Chief, Medical Neurology Branch, NINCDS OTHER: Valerie Askanas, M.D., Ph.D., Research Assistant Professor, Institute for Rehabilitation Medicine, New York University, New York, NY Steven P. Ringel, M.D., Chief, Dept. of Neurology, Univ. of Colorado Medical Center, Denver, CO Adam N. Bender, M.D., Assistant Professor of Medicine, Mt. Sinai Hospital, New York, NY Alberto L. Dubrovsky, M.D., Visiting Fellow, MN NINCDS N. Bojji Reddy, M.D., Visiting Associate, MN NINCDS John W. Gittinger, M.D., Clinical Associate, MN NINCDS					
COOPERATING UNITS (if any) Institute for Rehabilitation Medicine, New York University, New York, NY University of Colorado Medical Center, Denver, CO Mt. Sinai Hospital, New York, NY					
LAB/BRANCH Medical Neurology Branch					
SECTION --					
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20014					
TOTAL MANYEARS: 3.5		PROFESSIONAL: 1.5		OTHER: 2.0	
SUMMARY OF WORK (200 words or less - underline keywords) For a more clear identification of the association of the basic studies covered by this project with the various clinical diseases to which they relate, all aspects of this project have been incorporated into our on-going projects, numbers - Z01 NS 01034-14 MN Z01 NS 01039-14 MN Z01 NS 01189-08 MN Z01 NS 01190-12 MN					

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 00917-15 MN
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) Biochemistry Applied to the Study of Neurologic Disease		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: W. King Engel, M.D., Chief, Medical Neurology Branch, NINCDS Barry W. Festoff, M.D., Assistant Neurologist, MN NINCDS N. Bojji Reddy, M.D., Visiting Associate, MN NINCDS OTHER: Salvatore DiMauro, M.D., Assistant Professor of Neurology, Neurological Institute, Columbia-Presbyterian Medical Center, New York, NY Valerie Askanas, M.D., Ph.D., Research Assistant Professor, Institute for Rehabilitation Medicine, New York University, New York, NY Melvin Blecher, M.D., Professor of Biochemistry, Georgetown University School of Medicine, Washington, DC		
COOPERATING UNITS (if any) Neurological Institute, Columbia-Presbyterian Medical Center, New York, NY Institute for Rehabilitation Medicine, New York University, New York, NY Georgetown University School of Medicine, Washington, DC		
LAB/BRANCH Medical Neurology Branch		
SECTION --		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20014		
TOTAL MANYEARS: 4.7	PROFESSIONAL: 2.2	OTHER: 2.5
SUMMARY OF WORK (200 words or less - underline keywords) For a more clear identification of the association of the basic studies covered by this project with the various clinical diseases to which they relate, all aspects of this project have been incorporated into our on-going projects, numbers - Z01 NS 01034-14 MN Z01 NS 01039-14 MN Z01 NS 01189-08 MN Z01 NS 01190-12 MN		

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Myopathies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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LAB/BRANCH

Medical Neurology Branch

SECTION

--

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20014

TOTAL MANYEARS:

4.1

PROFESSIONAL:

2.6

OTHER:

1.5

SUMMARY OF WORK (200 words or less - underline keywords)

To more fully elaborate the clinical, histochemical, biochemical, ultra-structural, electrophysiologic and immunologic abnormalities of patients with the various myopathies. To further sub-classify patients in each category using those parameters. To seek pathogenic mechanisms, using a variety of different techniques including tissue culture and ones listed above, applied to the patient's body fluids and tissues, especially to the muscle biopsy specimens. To treat myopathic disorders by different methods in order to learn which is most effective within each disease category. To produce animal models of pathogenic phenomena.

Cooperating Units (Continued):

Neurological Institute, Columbia-Presbyterian Medical Center, New York, NY
Infectious Diseases Branch, NINCDS
VA Hospital, Washington, DC

Objectives: To more fully elaborate the clinical, histochemical, biochemical, ultrastructural, electrophysiologic and immunologic abnormalities of patients with the various myopathies. To further sub-classify patients in each category using those parameters. To seek pathogenic mechanisms, using a variety of different techniques including tissue culture and the ones listed above, applied to the patient's body fluids and tissues, especially to the muscle biopsy specimens. To treat myopathic disorders by different methods in order to learn which is most effective within each disease category. To produce animal models of pathogenic phenomena.

Methods Employed: A variety of techniques encompassing histochemistry, biochemistry, autoradiography, radionuclide scanning, electrophysiology, electronmicroscopy, tissue culture and immunology are applied to patients with the various myopathies and induced animal-models thereof.

Patient Material: Patients and diagnostic material from Medical Neurology Branch patients and from outside patients from whom diagnostic muscle biopsies were obtained and sent here for study.

Major Findings:

Myopathies are non-neurogenic, primary or secondary diseases of muscle. Some, such as the dermatomyositis/polymyositis group, are often at least partially treatable but their cause and details of their probably "dysimmune" pathogenesis are not known; others are not treatable but their cause is known, e.g., genetic deficiency of phosphorylase, phosphofructokinase, acid-maltase or carnitine-palmityl-transferase; while still others, such as Duchenne muscular dystrophy and others bearing the name dystrophy, are both of unknown pathogenesis and untreatable.

The polymyositis/dermatomyositis (PM/DM) disease-complex is an acquired disorder causing progressive deterioration of muscle in children and adults. The primary cause is not known but the pathogenic mechanism is considered "autoimmune" or "dysimmune". All patients were seriously incapacitated and many fatal before the introduction of anti-dysimmune therapy. Our studies involve improving diagnostic methods, seeking the cause and analysing the pathogenic mechanisms, and improving methods of treatment.

The special variation of anti-dysimmune treatment we introduced to this disease 8 1/2 years ago, long-term high-single-dose alternate-day prednisone (LT-HSDAD-Pred) has continued to be, in our series (about 60 cases) and others the single best available treatment for children and adults (without or with an associated cancer). It has the greatest benefit/side-effect ratio and is easiest to manage. However, because not all patients respond, we are defining some of the parameters of their immunologic response to prednisone and seeking predictive parameters thereof.

By use of T- and B-lymphocyte cell markers, T- and B-lymphocyte mitogens, and T-lymphocyte cytotoxicity on tissue-cultured chromium-labeled muscle fibers, we have now published that in PM/DM patients, while HSDAD-Pred is clinically cumulatively effective for months and longer, its measurable effect on the peripheral circulating lymphocytes, using currently available techniques, lasts less than 24 hrs; and these effects are more profound on the T-lymphocytes. We are now correlating these data with concurrently obtained blood levels of prednisone and prednisolone to determine any hypoabsorption or hypercatabolism of the drug (with P,CC). In patients, children and adults, inadequately responding to prednisone we have found on an occasional-case basis some remarkably therapeutic responses to added azathiaprine (3 mg/kg/d) -- to more clearly establish the efficacy of azathiaprine, a double-blind trial is being conducted. We are also measuring the patients' lymphocyte responses to azathiaprine.

Massive subcutaneous calcification can be a disabling complication of childhood DM. We have found new ways to clinically detect it in its early stages, isotopically with ^{99m}Tc -diphosphonate/body scanning (with NM,CC) and by xerography (for microscopic detection methods, v.i.) (with R,CC). However, our therapeutic trials with diphosphonate have not been beneficial (also not beneficial in a patient with myositis ossificans). However, in some severely affected patients the calcium has remarkably diminished as the muscle and skin were responding to our combined azathiaprine-HSDAD-Pred program. The reasons for this calcification are being sought by our histochemical, electronmicroscopic, and autoradiographic studies (v.i.). The exact mechanism of muscle damage in PM/DM is unknown. Previously we have reported immunoglobulin complexes deposited in blood vessels in 83% of the childhood cases and 29% of adult cases, findings supporting our earlier hypothesis that an aspect of muscle damage may be vascular. While disorders of both immunoglobulins (produced by B-lymphocytes) and direct immunocyte toxicity (T-lymphocytes) might be occurring in all cases of PM/DM, perhaps the former (deposited as intravascular immune complexes) are more muscle-damaging in the childhood form and latter are more pathogenic in the adult form. We are now attempting to correlate a possible vascular mechanism with our histochemical finding of preferential perifascicular involvement (occurring especially in childhood DM), as well as relating our findings in patients to our experimental animal model of muscle ischemia (v.i.). It is conceivable that the immunologic abnormalities of PM/DM are ultimately provoked by a viral infection; however, our attempts to see or "rescue" a virus from PM/DM patients' muscle directly after tissue culture of it, or after animal inoculation, thus far have been negative (with NYU, ID-NINCDS, and NCI).

A new and possibly disease-characteristic histochemical finding in PM/DM patients of microscopic foci calcium accumulation in collagenous connective tissue of muscle and subcutaneous regions has been demonstrated histochemically at both the light-microscopic (alizarin-red method) and EM (pyroantimonate method) levels. Its pathogenesis is being sought, as is its correlation with our positive ^{99m}Tc -diphosphonate patient-scans for calcium, our autoradiographs of patient skin and muscle biopsies for calcium, and our previously described highly-disease-characteristic histochemical finding of alkaline-phosphatase staining in the intramuscular connective tissue.

Duchenne muscular dystrophy (DMD) is an hereditary progressive deterioration of muscle in children, usually causing wheel-chair or bed confinement by age 12 and death by age 20 years. Cause and treatment are not known. Our studies involve improving diagnostic methods, seeking the basic cause, and trying experimental therapy when available. We also seek fuller understanding of the basic biology and pathologic responses of muscle. The other myopathies and the other neuromuscular diseases of unknown nature which we study are often devastating to children or adults, and in their study our general objectives are the same.

Current competing hypotheses of the pathogenesis of DMD are: (a) primary or secondary defect of blood supply to muscle, (b) primary defect of energy source within the muscle fiber, and (c) primary muscle-fiber membrane defect. Although many others favor c, we favor a or b. Some of the findings in DMD muscle considered by others to be supportive of c we consider to be invalid results or not distinguishing between c and b. The defect of erythrocyte morphology reported by others we are reporting as not present in our controlled studies. The defect of the muscle-fiber membrane evident by peroxidase penetration reported by others we find not disease specific. The alteration of muscle homogenate adenylcyclase reported by others we have reservations about because (i) it wasn't exclusive to DMD, and (ii) its cellular site (presumed by them to be muscle fiber sarcolemma) was not known from those biochemical studies. Our own newly developed histochemical method for adenylcyclase (AC) shows highest muscle AC in blood vessels and almost none detectable in normal muscle, but fairly high amounts of AC diffusely throughout the interior of regenerating fibers, especially evident in DMD biopsies.

Our new model involving the focal application of a mitochondrial poison to muscle fibers in situ, which results in the secondary loss of sarcolemmal membrane integrity of the damaged fibers, establishes an experimental basis for at least the possibility of hypothesis b, in harmony with our clinical demonstration that in muscle phosphorylase deficiency ischemic exercise precipitates acute breakdown of the sarcolemmal membrane barrier, allowing marked creatine-phosphokinase (CPK) egress and calcium ingress (detected by scanning with ^{99m}Tc -diphosphonate).

Our ischemia hypothesis for DMD, which proposes a functional defect on the arterial side of the vascular tree, has been based on our studies of the histochemopathology of DMD muscle and of our experimental ischemic myopathy in animals. As yet, an ischemia mechanism, although possible in DMD patients, has not been demonstrated in them directly. We are, though, now reporting that in known ischemic human muscle, caused by occlusive vascular disease, the pattern of histochemopathology of muscle (groups of necrotic or regenerating fibers in fields of normality) overlaps that of early DMD and of our experimental ischemic myopathy in animals, thus at least being harmonious with the ischemia hypothesis (with VAH, D.C.). A number of investigators are now attempting to evaluate our ischemia hypothesis. Although some feel they are disproving it, we have in a published formal forum provided counter-arguments of those criticisms suggesting that they are incorrectly based or inconclusive.

We used the ischemia myopathy model to study some of the details of the mechanisms of muscle fiber damage. One result was the development of a new, rapid and simple radioisotopic method, based on uptake of ^{99m}Tc -diphosphonate, of quantitating active skeletal muscle damage in experimental animals -- that uptake was directly correlated with other more arduously determined parameters of muscle damage, loss of muscle K^+ and elevation of plasma CPK (with AFRR1). We have now shown that those parameters are also directly correlated with uptake of ^3H -diphosphonate and with calcium uptake, the latter presumably the basis for the diphosphonate accumulations. Our autoradiography shows the uptake of ^3H -diphosphonate to be within muscle fibers, ones necrotic and even intact ones (presumably pre-necrotic or perhaps reversibly injured) -- this correlates very well (in histochemically-stained serial sections) with calcium accumulation within and complete loss of phosphorylase/glycogen staining from those fibers. We have shown that calcium accumulation in damaged muscle fibers is also evident by light- and electronmicroscopy-histochemistry in various human disorders -- it is not disease-specific but it is a new way to identify damaged fibers, including minimally affected ones not identifiable by other methods. Our electronmicroscopy-histochemical method (pyroantimonate) shows the calcium increase in the pathologic fibers to be in mitochondria > sarcoplasmic reticulum > myofibrils. Nuclei (nucleoli and heterochromatin) are also calciumed. These are the same organelles we find calciumed when normal intact muscle is soaked in a high calcium solution.

From all of these studies we have formulated a new hypothesis covering molecular aspects of muscle fiber damage, as follows: (a) any damage to the sarcolemma -- be it exogenous (e.g., trauma, ischemia), endogenous deficiency of energy supply (e.g., phosphorylase deficiency, mitochondrial poison) or, conceivable but not tested, a direct primary or secondary sarcolemmal defect -- can break the normal sarcolemmal barrier to calcium (Ca^{++} / Ca^{++} normally about 10,000-20,000/1); (b) the influxing Ca^{++} is taken up by those organelles known to normally have the greatest capacity to do so, i.e., mitochondria > SR > myofibrils, and nuclei; (c) this heavy calcium accumulation eventually can damage organellar function -- e.g., a high Ca^{++} is known to reduce mitochondrial ATP synthesis, which presumably would lead to further impairment of the sarcolemmal barrier and increasing damage of the muscle fiber; (d) the molecular death of the fiber may be due to the heavy calcium influx; if only mild influx recovery might occur.

Along with these theoretical and experimental data, we have demonstrated that patient scanning with ^{99m}Tc -diphosphonate can be used to detect abnormal uptake, presumably based on abnormal accumulations of calcium, in various disorders of muscle. There is: (a) greatest uptake in the polymyositis/dermatomyositis complex (probably enhanced by diffuse radiologically-invisible but histochemically and electromicroscopically-evident calcium in connective tissue of muscle and subcutaneous regions); (b) only moderate uptake in DMD even though the patients have very high serum CPK; (c) normal or minimally increased uptake in neurogenic involvement; (d) surprising moderately high uptake, in the presence of normal serum CPK's, in muscle of patients with ragged-red fibers (which contain abnormal mitochondria); (e) very high uptake in phosphorylase-deficiency muscle acutely damaged by ischemic exercise and in muscle acutely damaged by trauma (viz., at the site of muscle biopsy).

A survey chapter (>500 references) of the biology of the muscle cell in relation to myopathies has been written.

Other myopathies. Tissue culture of human muscle biopsies provides growing muscle fibers free of all neural, vascular, and humoral factors present in the patients. After we developed a new program (reported last year) for achieving abundant, reproducible and mature growth of human fibers in culture, including spontaneous twitching, and for precisely selecting certain fibers for enzyme histochemistry, immunohistochemistry, EM, or EM-histochemistry, we turned our attention to culturing the biochemically and the morphologically distinct myopathies.

In adult-onset acid maltase deficiency we have reported the first "reincarnation" in cultured muscle fibers of the biochemical and morphological defects characteristic of the disease -- the muscle fibers in the biopsy and the ones newly grown in culture (with NYU) were identical by histology (vacuoles), histochemistry (acid phosphatase-high in the vacuoles), electronmicroscopy (glycogen accumulated in lysosomes) and biochemistry (with Columbia) (absent acid maltase by the natural substrate method, 5-10% of normal acid maltase by the artificial substrate method, normal neutral maltase), normal kinetics of neutral and the trace-amount acid maltase, elevated acid phosphatase. This is the first establishment of a neuromuscular disease as a primary myopathy. It provides a new test system for manipulations directed toward the treatment or prevention of muscle-fiber damage in this disease without inducing any risk to the patient. Further, we have now similarly shown reincarnation of the severe biochemical and morphologic defects in 4 chronic-infantile cases of acid maltase deficiency and partial defects in 3 heterozygotes.

We recently reincarnated a second metabolically distinct myopathy, phosphofructokinase (PFK) deficiency. Extremely reduced levels of that enzyme were found in muscle fibers cultured from a patient with a new combined syndrome of muscle and erythrocyte PFK deficiency and hypolipoproteinemia (with NYU and Columbia). Growth of that muscle in culture was enhanced by B-hydroxybutyrate added to the medium, a ketone-body providing one of the alternate energy sources which that muscle must be depending on since its glucose-utilization pathway is defective (with NYU).

In a third glycogenolytic-enzyme defect of muscle, phosphorylase deficiency, we have confirmed our previous finding that the enzymatic absence of the muscle fibers is "cured" with their growth in culture -- actually the remarkable and unexpected recovery of phosphorylase activity is seen in fibers in the regenerative state in culture and in vivo (with NYU). The therapeutic thrust need now is to provoke appearance of that phosphorylase in the mature fibers of the patient.

A morphologically characteristic defect, "cabbage bodies" (multi-lamiated material in lysosomes) in muscle fibers of a patient with a chronic myopathy, were reproduced in his cultured muscle fibers (with NYU). Although the biochemical defect is not known, both the biopsied and cultured muscle had elevated acid phosphatase, suggesting the patient has a defect of a

yet-unpinpointed lysosomal hydrolytic enzyme (with NYU and Hôpital des Enfants Malades, Paris). We are assaying 12 different lysosomal enzymes in our cultures of human and animal muscle to search for defects thereof (with NYU and Hôpital des Enfants Malades, Paris).

"Ragged-red" muscle fibers, which contain severe mitochondrial abnormalities, are the commonest histochemical accompaniment in limb muscles of the mixed syndrome of progressive external ophthalmoplegia in our series of 41 patients (after myasthenia gravis and myotonic atrophy are excluded). Some, though not all, of those mitochondrial changes have been reincarnated in limb muscle cultured from such patients (with NYU) -- the mitochondrial crystal-like inclusions have not been. In the original biopsies those mitochondrial inclusions usually lacked cytochrome oxidase staining by our EM-cytochemistry.

In tissue cultures of "normal" chick embryo skeletal muscle we were able to induce certain bizarre changes of mitochondrial morphology by pulses of dinitrophenol (DNP), but did not produce all the mitochondrial changes occurring in the human "ragged-red" fibers (with NYU). In the course of that study we found that DNP markedly promoted the detectability of avian oncornavirus (the now-preferred name for the group which includes leucosis and Rous sarcoma viruses) in all such "normal" chick muscle cultures -- virus was evident morphologically (C-particles) and by complement fixation titers (COFAL) (with NYU). This demonstrated that the "normal" chick embryo muscle cultures so frequently used by various investigators for physiologic, biochemical, immunologic and developmental studies are actually virally-contaminated test objects. The question of whether that oncornavirus has a "normal" role in development of "normal" chick muscle in vivo or in vitro is raised by this study. Our finding the C-particles exclusively in dilations attributed to T-tubules raises the intriguing possibility that T-tubules might be the aqueducts of virus infestation of or shedding from muscle fibers. The question of viruses harbored in mitochondria (as "mitochondriophages", analogous to bacteriophages) is raised by the C-particles being provoked by DNP, an uncoupler of mitochondria oxidative phosphorylation. Finally, we have raised the speculation that such mitochondriophages might be responsible for the changes in the ragged-red-fiber diseases.

A new type of mitochondrial abnormality, light-cored dense particles, has been reported in skeletal muscle fibers of a patient with cardiac "asymmetric septal hypertrophy". A new neuromuscular disorder, striped loss of mitochondria, has been found in a family with dominantly inherited distal muscle weakness. In biopsies of 3 patients with sporadic chronic myopathies we have by electronmicroscopy found under the plasmalemma long strands suggesting viral material. In a patient with late-onset rod disease, an entity first described by us a few years ago, an associated unusual serum paraprotein, IgG/lambda myeloma protein has been found. Its significance is not known.

We have now reported in 3 cases a new clinically-delineated "levitated arms syndrome" and identified the cause as muscle fibrosis resulting from iatrogenic intramuscular injections into the deltoids, especially of pentazocine (Talwin). One patient also had levitated legs (evident when sitting) from pentazocine injections into the rectus femoris muscles. Two were successfully treated by surgical transection of fibrous bands, in the deltoids of both and also the recti femoris in one. These cases demonstrate a newly-recognized, preventable, and treatable iatrogenic disease.

Neuromuscular disorders of uncertain classification. Selective atrophy of the type-II (glycolytic-rich, oxidative poor) muscle fibers, especially the subtype IIB fibers, has been shown to be the basis of cachectic atrophy accompanying cancer and other chronic debilitating disorders. We have now reported a detailed analysis of this phenomenon and formulated possible hypothetical mechanisms. They are: (a) a "Sparafucile" factor (v.i.), directly or indirectly resulting from the cancer, indiscriminately assassinating the type-II fibers, vs. (b) a muscle-fiber martyrdom mechanism of protein catabolism to supply energy substrate, via the alanine shunt and glyconeogenesis, to cells more vital to the organism. The possible neurogenic vs. myogenic pathogenesises of the type-II atrophy have been analysed and theoretical models based on empiric findings constructed. Evaluation of the mechanism of type-II fiber atrophy in cancer patients is important because this "remote effect" muscle weakness is often the most crippling aspect of cancer -- if the molecular mechanism can be discovered it might be treatable independently of treatment and response of the cancer itself. And improvement of the muscle weakness and wasting could even make the patient better able to withstand the rigors of direct anti-cancer therapy.

Malignant hyperthermia (MH) is a syndrome, 70% fatal, of acute rise of body temperature and muscle rigidity during general anaesthesia. A number of the patients have underlying not-well-defined neuromuscular disorders. In one MH patient, and his father, we have identified central-core disease (CCD), with its typical type-I muscle fiber predominance (with Children's Hospital, Wash., D.C.). Because there are in the literature two other families with CCD and MH, we have issued a caution to all our CCD patients and their physicians regarding the possibility of MH during general anaesthesia. The mechanism(s) of the attack of MH is not known. It appears that there is an excess of free intracellular calcium in the muscle fiber, which we propose might be due to an effect of the anaesthetic or muscle-relaxant agent on the calcium-barrier function of the sarcolemma (v.s.). We are now investigating why central core disease, which we have earlier postulated to be due to a congenital monophasic neuropathy mainly affecting the type-II units, should predispose to the development of MH.

Myotonic atrophy (myotonic dystrophy) is of unknown pathogenesis. We have previously raised the possibility of at least partially a neurogenic aspect. To evaluate myotonic phenomena by various parameters we have set up the animal model of 20,25 diazacholesterol-induced myotonia (originally described by others). Although the animals become markedly myotonic, we find that their muscle reveals no histochemical alterations.

The systemic approach in application of a rapid short (5-stain) and long (18-stain) battery of histochemical reactions to fresh-frozen sections of human muscle biopsies (including basic stains developed, e.g., our modified trichrome) and the concepts in the analytical approach to neuromuscular pathology we have developed are being followed in nearly all centers for neuromuscular research throughout the world.

Cross-category and basic aspects. Previously we have formalized and seen adopted by the World Federation of Neurology our long-used nomenclature for histochemical types of human limb-muscle fibers, based on two types, I and II. One of our former trainees has shown histochemical subtypes of the type-II fibers. Now we have published histochemical distinction of two subtypes, IA and IB, of the type-I fibers and shown selective involvement of a subtype in certain human neuromuscular disorders (with NYU).

Eye muscle fibers histochemically are not exactly like limb muscle fibers. We have demonstrated their normal histochemical patterns in Rhesus monkey and identified 3 types, "fine", "granular" and "coarse". The first two types have one endplate per fiber and probably are types of twitch fibers; the last has multiple endplates and may be a tonic fiber. Following denervation the first two developed extrajunctional acetylcholine receptors but the coarse fibers did not -- no fibers were positive beyond 13 weeks post-denervation.

The autoradiography laboratory was established in our own Branch this year. We have begun with 3H -diphosphonate to demonstrate intracellular calcium (v.s.). Now we are developing a technique for doing autoradiography of biopsy samples following injection of some of the short-lived gamma-emitting radionuclides used in patient scanning, to establish direct scanning-histoautoradiographic correlations.

Mammalian sarcolemmal membranes have received major biochemical emphasis. Results of the following studies are now being published. Not only have pure fractions of rat sarcolemma been obtained, but methods have been perfected that provide adequate quantities of sarcolemmal membrane from human muscle obtained from limb amputation or radical mastectomy. With the sarcolemmal membrane fractions and subfractions, methods have been established for studying acetylcholine receptor, acetylcholinesterase, $Na^{+}-K^{+}$ ATPase (Na^{+} -stimulated phosphorylation), $adenylcyclase$, divalent cation (viz., Ca^{++}) binding/transport, and Ca^{++} -stimulated ATPase. Some of these have been studied in sarcoplasmic reticulum fractions as well. Elucidated have been detailed properties of: (a) the $adenylcyclase$ (i.e., fraction localization, kinetics, catecholamine-activation, guanylyl-inidodiphosphate activation, insulin and glucagon inhibition, and response to denervation in "red" cf. "white" muscle of animals); (b) the sarcolemmal protein phosphorylation (i.e., Na^{+} enhancement blocked by K^{+} , phosphorprotein state suggesting an acylphosphate bond, high turnover rate suggesting it is a functional intermediate of $Na^{+}K^{+}$ ATPase, and molecular weight); (c) the effect of denervation on this transport ATPase system; (d) the Ca^{++} uptake and release by human sarcoplasmic reticulum (SR) (i.e., by use of specific antibodies made against SR which block the Ca^{++} uptake and inhibit $adenylcyclase$ but do not affect ATPase activity, suggesting

different localization of these functions within the SR); (e) the role of bound-calcium in the regulation of Ca^{++} transport by SR; and (f) physicochemical properties of ACh-receptor cf. acetylcholinesterase. These assays are now being used to seek biochemical defects of sarcolemmal or sarcoplasmic reticulum function in muscle biopsies from patients with various neuromuscular disorders.

Insulin receptor function is being analysed in our cultured chick muscle fibers as the first step toward studies of diabetic neuropathy patients (with Georgetown University). The effect of dibutyl cyclic AMP and a cAMP-phosphodiesterase inhibitor (phthalazinol) on growth and maturation of human and animal cultured muscle fibers is being evaluated (with NYU).

Significance to Bio-Medical Research and the Program of the Institute: These findings provide new information on the pathologic and pathogenic aspects of the various myopathies, on the treatment of some, and on animal-models of some of the myopathies.

Proposed Course of Project: The studies underway are part of a long-term project consisting of interrelated studies which will continue for several years.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01037-14 MN

PERIOD COVERED July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Tissue Culture Applied to the Study of Human Neurologic Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: W. King Engel, M.D., Chief, Medical Neurology Branch, NINCDS
Valerie Askanas, M.D., Ph.D., Research Assistant Professor, Institute for
Rehabilitation Medicine, New York University School of Medicine,
New York, NY

COOPERATING UNITS (if any)

Institute for Rehabilitation Medicine, New York University School of Medicine,
New York, NY

LAB/BRANCH

Medical Neurology Branch

SECTION

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INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20014

TOTAL MANYEARS:

2.4

PROFESSIONAL:

1.2

OTHER:

1.2

SUMMARY OF WORK (200 words or less - underline keywords)

For a more clear identification of the association of the basic studies covered by this project with the various clinical diseases to which they relate, all aspects of this project have been incorporated into our on-going projects, numbers - Z01 NS 01034-14 MN
Z01 NS 01039-14 MN
Z01 NS 01189-08 MN
Z01 NS 01190-12 MN

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01039-14 MN
PERIOD COVERED <p style="text-align: center;">July 1, 1975 through June 30, 1976</p>		
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Amyotrophic Lateral Sclerosis (ALS) and Other Lower Motor Neuron Diseases</p>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: W. King Engel, M.D., Chief, Medical Neurology Branch, NINCDS Benjamin R. Brooks, M.D., Clinical Associate, MN NINCDS OTHER: John W. Griffin, M.D., Assistant Professor of Neurology, Johns Hopkins School of Medicine, Baltimore, MD Donald L. Price, M.D., Associate Professor of Neurology & Neuropathology, Johns Hopkins School of Medicine, Baltimore, MD John L. Sever, M.D., Chief, Infectious Diseases Branch, NINCDS David S. Zee, M.D., Assistant Professor of Neurology & Ophthalmology, Johns Hopkins School of Medicine, Baltimore, MD David G. Cogan, M.D., Medical Officer, IR NEI Robert D. Yee, M.D., Clinical Associate, CB NEI Jonas E. Sode, M.D., Chief, Endocrinology Branch, NMMC, Bethesda, MD John W. Gittinger, M.D., Clinical Associate, MN NINCDS T. Shimamoto, M.D., Director, Institute of Japan Atherosclerosis Research Foundation, Tokyo, Japan		
COOPERATING UNITS (if any) Johns Hopkins School of Medicine, Baltimore, MD Infectious Diseases Branch, NINCDS Intramural Research and Clinical Branch, NEI (see below)		
LAB/BRANCH <p style="text-align: center;">Medical Neurology Branch</p>		
SECTION <p style="text-align: center;">--</p>		
INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, MD 20014</p>		
TOTAL MANYEARS: <p style="text-align: center;">1.8</p>	PROFESSIONAL: <p style="text-align: center;">1.6</p>	OTHER: <p style="text-align: center;">0.2</p>
SUMMARY OF WORK (200 words or less - underline keywords) <p>In ALS and other diseases affecting the lower motor neurons, we are seeking (a) more precise morphologic and chemical definition of the abnormalities; (b) separation of each disorder into more distinct, and often new, sub-forms; (c) specific or symptomatic therapeutic response; (d) new methods of analysing the abnormalities; and (e) animal models of the human patho-physiologic states.</p>		
Cooperating Units (Continued) : Endocrinology Branch, National Naval Medical Center, Bethesda, MD Institute of Japan Atherosclerosis Research Foundation, Tokyo, Japan		

Project Description:

Objectives: In ALS and other diseases affecting the lower motor neurons, we are seeking (a) more precise morphologic and chemical definition of the abnormalities; (b) separation of each disorder into more distinct, and often new, subforms; (c) specific or symptomatic therapeutic response; (d) new methods of analysing the abnormalities; and (e) animal models of the human pathophysiologic states.

Methods Employed: A variety of techniques, encompassing histochemistry, biochemistry, autoradiography, radionuclide scanning, electrophysiology, electronmicroscopy, immunology and tissue culture are applied to patients with the various diseases covered in this category and to induced animal-models thereof. Conducted were double-blind, single-blind and non-blinded therapeutic trials, the efficacy of which is judged by clinical testing, functional evaluation, and serial quantitative evaluation of muscle function using an apparatus designed by us for quantitating isometric muscle tension.

Patient Material: Medical Neurology Branch patients and neurology consultation patients from the various services of the Clinical Center, who had diagnostic muscle biopsy and other diagnostic procedures.

Major Findings:

Amotrophic lateral sclerosis (ALS) is a progressive neurologic disease affecting motor neurons, of adults, usually leading to death within 1-3 years of onset. The cause and treatment of ALS are unknown. Our studies involve improving diagnostic methods, delineating subtypes of ALS and other possibly treatable diseases mimicing ALS (several cases of which we have found in the past year), seeking the basic cause and trying experimental therapy when available. We also seek fuller understanding of the basic biology and pathologic responses of the motor neurons. We have especially pursued a possible metabolic cause, but have also looked for evidence of viral causation.

Detailed, repeated and reproducible radioimmunoassay studies of more than 125 patients have revealed that in ALS patients there is a distinctly low cerebrospinal fluid (CSF) level (compared to paranormals and myopathy and polyneuropathy disease-controls) of cAMP, and a slight decrease of cGMP (with Naval Medical Center). I.V.-probenecid studies demonstrate this is due not to increased clearance of cAMP or cGMP from CSF but probably to decreased synthesis in the neural tissue (or perhaps increased breakdown, or both). The low levels of cAMP, but not of cGMP, were corrected with a phosphodiesterase inhibitor, phthalazinol, which had no effect on plasma cAMP and cGMP (with Institute of Japan Atherosclerosis Research Foundation). The significance of this newly recognized biochemical defect in the pathogenesis of ALS remains unknown. We have shown, in ALS and non-ALS patients, that cAMP in plasma does not cross the blood-brain barrier during acute elevations -- glucagon raised plasma cAMP 40-fold and urine cAMP rose, but there was no change of cAMP in CSF; no change in cGMP of plasma or CSF followed glucagon.

Thus, CSF levels of cAMP reflect only metabolism of it within the central nervous system. The glucagon caused a 2-fold rise of blood glucose and a concomitant rise in CSF glucose. CSF cAMP and cGMP are somewhat elevated in patients with spasticity of various causes (excluding ALS).

The cells from which cAMP in the CSF arises are not known. We have developed a new technique for histochemically demonstrating adenyl cyclase, the enzyme synthesizing cAMP. It shows, in the spinal cord, cerebellum, brain stem and cerebellum, that the greatest amount is in the blood vessels with much lesser amounts in neural or glial tissue; almost none occurs in purkinje cells, while anterior horn motor neurons have slight to moderate amounts. A large amount was also in the epithelial cells of the choroid plexus and in the pineal cells. In lower motor neurons (LMNs) cAMP could, if its presence there is significant, conceivably have some role in excitatory or inhibitory transmission, in proximally or remotely generated trophic effects, and/or in internal metabolism such as in the rich glycogen metabolism machinery (that we have previously demonstrated to be a special characteristic of LMNs and cortical motor neurons and which, therefore, could conceivably be the site of the defect causing ALS).

Previously we have reported a neurologic abnormality with certain features like ALS in many cases of primary and secondary hyperparathyroidism; we have also studied a few cases with both frank hyperparathyroidism and frank ALS. To study the possible role of abnormality of parathyroid function and/or calcium metabolism in ordinary ALS, we have done radioisotopic calcium retention tests (n=80) on ALS patients and disease-controls: we have found abnormally low calcium retention in 63% of ALS, 60% of polyneuropathy, and only 35% of myopathy patients (with NNMN). So far, treatment with 20,25 dihydrotachysterol has been found to reverse the calcium-retention defect in some ALS patients but not result in clinical improvement.

Phthalazinol treatment of ALS patients, although able to raise their low CSF cAMP, has not resulted in consistent therapeutic benefit to date.

In ALS patients, including those with a late-post-polio progressive muscular atrophy syndrome, we continue to search for evidence of a viral cause in ALS sera, CSF and tissues by viral antibody profiling (polio 1,2,3, mumps, measles, rubella, coxsackie, and influenza A and B (with ID, NINCDS)), and various tissue culture techniques (including activation techniques and evaluation by morphologic, antigenic, and reverse transcriptase assays). To date no viral evidence has been found. Nevertheless, an animal model of reactivation of a latent virus infection was established -- latent infection of mouse dorsal root ganglia with herpes simplex virus type 2 or 1 could be reactivated by injuring the peripheral nerve (with NIDR). Evaluation of HLA types has not revealed any predominant type represented in our ALS patient group.

From our cumulative experience with more than 500 patients with ALS or ALS-like syndromes we emphasize that (a) about 10 are chronic, surviving more than 5-15 years (and therefore giving false hope to experimental therapists if they are not aware of this subgroup), and (b) as many as 15% or so of the cases referred to us as ALS have a different disease, more benign, and occasionally treatable -- they are identified by careful clinical and laboratory examinations.

Two extensive chapters have been written, one on numerous aspects of the biology of the lower motor neurons as a basis for understanding and investigating diseases thereof (>1000 references), and the other on various aspects of the several motor neuron diseases (>300 references).

Previously we have introduced the new concept, on the basis of our combined electromyography (EMG) and histochemistry studies, our open-biopsy-EMG technique, and on theoretical grounds, that an EMG pattern on voluntary effort of brief-duration small-amplitude overly-abundant motor-unit action potentials (BSAPs), sometimes polyphasic (BSAPPs) is just as possibly a reflection of neuropathic as of myopathic diseases -- and we criticized the established (and we would say vacuous cliché of "myopathic EMG" for that pattern. Initially not met with enthusiasm by hard-core electromyographers, our hypothesis is now becoming accepted by at least some of them, as well as by many less tradition bound investigators.

Altered influence of the lower motor neuron on muscle fibers was identified and studied by use of the new α -bungarotoxin immunoperoxidase technique at light-microscopic and EM levels for identifying acetylcholine receptors (AChRs). Especially useful was the visualization of the AChRs when present diffusely in the plasmalemma following denervation. Last year we demonstrated that the diffuse extrajunctional AChR can be used for identifying and "dating" denervated fibers. This year we have used it to look for evidence of altered neural influence in several diseases not generally considered neurogenic but ones in which we have previously postulated possibly to be on a neurogenic rather than a myopathic basis -- they include central core disease, type-I-fiber hypotrophy with and without central nuclei, myotonic atrophy, myotonia congenita, some cases of benign congenital hypotonia, some of type-II-fiber atrophy, and congenital and adult-onset rod diseases. None of these disorders showed extrajunctional AChRs by light-microscopy -- however, the question is still not fully resolved because: (a) there could be slight extrajunctional α BBT binding that is demonstrable only by electron-microscopy, or (b) there could be in some of those diseases a subtle incomplete neurogenic alteration of neural influence partially affecting muscle fiber function and morphology without allowing extrajunctional AChR to develop in those fibers.

Conversely, a new concept of "myogenous mal-innervation" was formulated. It was based on our demonstration that: (a) diffuse extrajunctional ACh-receptors were present on "regenerating-degenerating" muscle fibers in myopathic biopsies of our patients, (b) human, rat, and chick embryo skeletal muscle

grown in tissue culture without innervation (pre-innervated muscle) has diffuse extrajunctional ACh-receptors (with NYU), and (c) in adult animals, segments of muscle fibers experimentally separated from their motor innervation point but remaining viable develop diffuse extrajunctional ACh-receptors. Thus we have formulated a "law of mal-innervation", since it becomes evident that the presence of diffuse extrajunctional AChR in muscle fiber plasmalemma is certainly a sign of mal-innervation of the muscle fiber but it is not pathogenesis-specific since it can be either a neurogenous or a myogenous de-innervation or dys-innervation, or a primary non-innervation.

The neurogenously denervated fibers in ALS-patient biopsies with demonstrable extrajunctional receptors were used as the more sensitive assay for finding the blocking factor in sera of myasthenia gravis patients (v.s.).

In patients with scoliosis, our studies have continued to show a wide variety of neuromuscular diseases (by muscle biopsy histochemistry and electromyography) to be associated with and probably causing scoliosis. The most common pathology is some form of neurogenic muscular atrophy. Some of our scoliosis cases were previously considered "idiopathic". We therefore are embarking on a broad collaborative survey to define any underlying neuromuscular pathology in all available cases of "idiopathic scoliosis".

We have utilized new techniques for identifying T- and B-lymphocytes in human cerebrospinal fluid in more than 100 patients. The mean percents of total CSF lymphocytes are, respectively, 72 and 16 (12 being null cells). Changes in various diseases are being investigated, as are the responses to prednisone.

Polyneuropathy (Peripheral Neuropathy) (PN): The peripheral neuropathies comprise a group of disorders of various causes, more than half unknown. They always cause serious physical handicap sooner or later in the course of the disease, sometimes associated with intractable pain and ulceration and loss of feet and hands. Our studies seek to delineate the underlying causes and where possible develop a treatment. We also seek fuller understanding of the basic biology and pathologic responses of the lower motor and sensory neurons and peripheral nerves.

The majority of patients we see are of undiscernable cause. Those which are non-familial we may treat with LT-HSDAD-prednisone, especially if less than 5-years duration. We have had good to outstanding success in more than 25 such patients, most having been given-up on by others and some having come with diagnoses of non-treatable diseases (e.g., ALS or "Charcot-Marie-Tooth" disease). Long-term treatment is required -- too-rapid reduction of dosage too soon often results in exacerbation of disease. Excellent results have been sustained for as long as 11 years in an adult and 8 1/2 years in a child (who at age 20 is still regaining motor skills). Our correlative studies indicate that patients most likely to respond are dysschwannian in type (slow nerve-conduction times), relapsing, with elevated CSF protein, but even some non-relapsing patients without slowed nerve conduction times and with normal CSF have responded to LT-HSDAD-pred.

An often undiagnosed cause of sensory-greater-than-motor neuropathy beginning in adulthood is "idiopathic" amyloidosis. We propose the combination of crystal violet stain with fresh-frozen sections of a muscle biopsy (proven by us by a decade of use) as the choice method of diagnosing this disorder. In our most recent 10 cases of non-familial amyloid polyneuropathy, the onset was in later adulthood (mean age 54); 8 were male. We emphasize that this disorder is associated with, and probably the result of, a plasma cell dyscrasia, detectable in 8/10 of our patients as multiple myeloma, and/or serum and/or urine "paraprotein" immunoglobulin fragments. We propose that the neuropathy is due to a systemic metabolic abnormality, possibly related to a circulating abnormal protein fragment (i.e., a Sparafucile phenomenon, v.s.), rather than to pressures from multifocal "amyloid" deposits of immunoglobulin fragments. Our treating 6 amyloid patients with melphalan, an "anti-myeloma" agent, has not been of obvious value. A radioisotope scanning method has proved to be a new diagnostic technique for identifying amyloid in soft tissues -- highly suggestive of this diagnosis is (in the presence of a normal soft-tissue x-ray) a diffusely positive scan, presumably based on binding of the tracer to cations that are binding to the very anionic amyloid fibrils, a mechanism supported by our EM histochemistry studies of amyloid.

We have recently established combined studies of patient nerve biopsies in vitro -- including in vitro nerve conduction velocities of fast and slow fibers, teased fiber histochemistry, electronmicroscopy, and EM-histochemistry (with autoradiography to be added imminently), allowing more precise and direct multidimensional analyses of the afflicted nerves in polyneuropathy patients.

Anterograde axoplasmic transport of protein, glycoprotein and other substances from the neuron soma to the periphery is critical in maintaining integrity of the peripheral nerves. There is also a retrograde axonal transport, the normal role of which is uncertain but, we hypothesize, may serve for: (a) continuous monitoring by the soma of its distal self (e.g., regarding the necessity or not for axonal regeneration), (b) monitoring the external environment of its tip, and (c) acquisition of nutrients; it may also serve as the mechanism by which toxic substances are acquired (v.i.). Our autoradiographic studies (with Johns Hopkins) have demonstrated in animals a rapid anterograde transportation to the axon synaptic terminal at the neuromuscular junction and accumulation there of large amounts of protein and glycoprotein synthesized in the lower motor neuron soma from precursors leucine and fucose injected into the ventral horn less than 24 hrs. earlier. This demonstrated that these rapidly transported axonal proteins and glycoproteins go rapidly to the geographic location where they can be used, hypothetically, for (a) remodeling of the axonal tips and (b) trophic influence on muscle -- both possibilities we are studying further. Combined electronmicroscopy and autoradiography demonstrated that ligation of the nerve interrupted both anterograde and retrograde rapid axonal transport, causing intra-axonal accumulation of masses of smooth vesicular membranes on both proximal and distal sides of the ligation and of substances labeled from precursors injected centrally or peripherally respectively. Those results suggest that fast anterograde and retrograde axonal transport are very similar

processes carrying predominantly membranous organelles and constituting a system of bidirectional fast transport (which is interrupted focally by the nerve ligation). Studies of axonal transport provide a means for investigating the origin and fate of axonal organelles in pathologic processes. Our autoradiographic studies have also shown: (a) direct evidence for retrograde intra-axonal transport of tetanus toxin, and (b) that fast anterograde axonal transport provides a necessary contribution to motor nerve regeneration in experimentally sectioned nerves.

A new principle/model for inducing an experimental allergic neuropathy (EAN) in animals has been demonstrated. It involves immunization with soluble nerve protein (in contrast to lipid-associated protein of myelin used in previous EAN models). This represents a new potential model of some human dysimmune dysneuronal peripheral neuropathies, such as in some patients we have seen with prednisone-responsive neuropathies without demonstrable schwann-cell involvement. It also represents a new approach to studying certain dysimmune disorders of the CNS, such as multiple sclerosis and parainfectious encephalopathies. Since our EAN animals also have a component of blockade of neuromuscular transmission that is responsive to edrophonium, the model may have some relevance to myasthenia gravis or other disorders of the neuromuscular junction.

The group of spinocerebellar degenerations comprises diseases of various causes, a few known, most not. They always result in serious physical handicap sooner or later in the course of the disease, and sometimes mental deterioration. Our studies seek to delineate the underlying causes, and where possible attempt to develop a treatment. Previously, we have delineated some specific disorders within this group, e.g., acanthocytosis-with-normal lipoproteins and pyruvate decarboxylase deficiency (ataxia intermittent), and defective oxidation of puruvate in some patients with the Friedreich's ataxia sub-syndrome. Now we have identified a patient with spinocerebellar degeneration as having "sea-blue histiocytosis". In one of our familial cases of slowly progressive dementing spinocerebellar degeneration syndrome fatal at age 17, we discovered intraneuronal storage material, some lipofuscin-like and PAS-positive and some PAS-negative. This material has now been identified by others as G_{M2} -gangloside.

cAMP is thought by some to be a mediator of synaptic transmission of some systems in the cerebellum. Our newly developed histochemical technique for demonstrating adenyl cyclase (AC), its synthesizing enzyme, shows the greatest amount to be in cerebellar blood vessels, especially those adjacent to the bases of the purkinje cells, while the purkinje cells themselves are nearly negative. From this study a concern is raised regarding the possible influence of blood-vessel AC and cAMP in biochemical assays of homogenates of microdissected tissue samples.

Some patients with spinocerebellar degeneration have slow eye movements. It was found that they made abnormally slow refixational eye movements by the saccadic system (i.e., they were slow saccades) rather than by the voluntary pursuit system. This has led to the proposal of a new conceptual scheme of how both normal and defective saccadic eye movements might be generated. Study of a group of related patients with familial late-onset cerebellar ataxia has revealed new information about non-visual control of eye position, since the striking abnormality was a defective smooth-pursuit and fixation system. The patients showed evidence of various non-visual mechanisms of maintaining eye position that have not been previously delineated. Rebound nystagmus was shown to occur in normal individuals if fixation is eliminated -- however it becomes clinically apparent in patients with spinocerebellar degeneration because of a coexisting defect of visually-mediated fixation mechanisms. Thus rebound nystagmus can be interpreted as a manifestation of one of the brain's compensatory mechanisms for maintaining eye position when visual systems are ineffective.

Progressive spastic paraplegia is a progressively crippling disorder of children and adults. The causes are not known. Identified were three unrelated patients with a syndrome of chronic adrenal insufficiency from infancy and juvenile-onset of progressive spastic paraplegia and "onion-bulb" peripheral neuropathy, with normal intelligence (with NIAMDD). A single metabolic defect is postulated to underlie the abnormalities in the neural and adrenal tissues (? an adrenoleucodystrophy variant).

Ophthalmoneurology: We have shown that the various neuromuscular disorders affecting the eyes, if correctly evidenced by their limb-muscle pathology, can be on a neuropathic or myopathic basis. They cause various degrees of handicaps. Our studies seek to delineate the underlying disorder, analyze the neuro-ophthalmologic defect, and, if possible, seek methods of treatment. We also seek fuller understanding of the basic biology and pathologic responses of the eye neuromuscular apparatus. The commonest associated limb-muscle pathology of the progressive external ophthalmoplegia syndrome (in our series of 41 patients, after myasthenia gravis and myotonic atrophy are excluded) is a syndrome characterized by "ragged-red" muscle fibers in limb muscles, whether or not the limbs themselves are weak. Those ragged-red fibers contain mitochondrial abnormalities of various types. We have partly but not completely re-incarnated those mitochondrial changes in muscle tissue-cultured from such patients; and we have produced mitochondrial changes in cultured normal chick muscle by dinitrophenol (v.s.) (with NYU). Other limb muscle pathology associated with progressive external ophthalmoplegia in our cases includes (i) a vacuolar myopathy plus neuropathy, (ii) type-I-fiber hypotrophy with central nuclei, (iii) only denervation, (iv) only morphologically nonspecific myopathy, and (v) type-II-fiber smallness. The histochemical fiber-typing of normal animal eye muscles and the different post-denervation response of extrajunctional receptor distribution of those fiber-types was shown (v.s.). Abnormalities of eye movement in the spinocerebellar ataxias and in myasthenia gravis are discussed above.

EM-histochemistry for calcium (with NEI) has shown its discrete localization within the sacs of the outer segment of the rod cells of the retina. This suggests that the sacs may have a function of calcium storage-and-discharge with photoexcitation analogous to that of the sarcoplasmic reticulum of muscle. The finding has importance in the hypotheses of photo-electrical coupling mechanisms.

Progressive blindness was described in two children with acute lymphocytic leukemia intensively treated with chemotherapy and radiation and considered a possible complication of that therapy (with NEI).

Significance to Bio-Medical Research and the Program of the Institute: These findings provide new information on the pathologic and pathogenic aspects of the various lower motor neuron disorders, peripheral neuropathies, and spinocerebellar degenerations, on the treatment of some, on animal-models of some of these disorders.

Proposed Course of Project: To more fully develop the interlinked basic and clinical studies underway directed toward clarification of the pathogenesis and identification of the etiology, and toward elaboration of means of treatment and prevention of these disorders.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01189-08 MN

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Episodic Weakness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: W. King Engel, M.D., Chief, Medical Neurology Branch, NINCDS

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COOPERATING UNITS (if any)

None

LAB/BRANCH

Medical Neurology Branch

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NINCDS, NIH, Bethesda, MD 20014

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0.2

PROFESSIONAL:

0.2

OTHER:

0.0

SUMMARY OF WORK (200 words or less - underline keywords)

To define more clearly and to treat those disorders affecting the neuromuscular apparatus which present primarily with episodic weakness or paralysis. Attention is directed toward those conditions in which evidence suggests that the main site of intermittent dysfunction is somewhere within the following portions of the muscle fiber: sarcolemma, T-system, sarcoplasmic reticulum, myofibrillar complex (i.e., the total excitation-contraction coupling mechanism). Studies are done with agents which are either provocative or therapeutic with respect to periodic paralysis syndromes, with a view to obtaining more information regarding abnormalities of pertinent metabolic pathways and methods of treatment.

Project Description:

Objectives: To define more clearly and to treat those disorders affecting the neuromuscular apparatus which present primarily with episodic weakness or paralysis. Attention is directed toward those conditions in which evidence suggests that the main site of intermittent dysfunction is somewhere within the following portions of the muscle fiber: sarcolemma, T-system, sarcoplasmic reticulum, myofibrillar complex (i.e., the total excitation-contraction coupling mechanism). Studies are done with agents which are either provocative or therapeutic with respect to periodic paralysis syndromes, with a view to obtaining more information regarding abnormalities of pertinent metabolic pathways and methods of treatment.

Methods Employed: Various techniques of clinical investigation (including electromyography and clinical biochemistry), muscle biopsy with samples for histochemical analysis, electromicroscopy, and biochemical assays of tissue were utilized. Provocation loading tests and therapeutic trials to raise or lower potassium or sodium were used. Acetazolamide was administered as a prophylactic agent for hypokalemic periodic paralysis.

Patient Material: Patients of all ages are admitted to the Medical Neurology Branch for this project if they have: intermittent muscular weakness associated with familial periodic paralysis, hypo- or hyperkalemic; isolated examples of periodic paralysis with potassium disturbances; thyrotoxic periodic paralysis; paramyotonia congenita; or myotonia congenita (Patients with myasthenia gravis are part of another Medical Neurology Branch project by that name.)

Major Findings: Periodic paralysis (PP) are hereditary or acquired disorders causing chronic weakness punctuated by attacks of paralysis. Associated metabolic abnormalities are known but the actual pathogenic mechanisms are not. Standard palliative preventive therapy in the idiopathic hypokalemic form of PP is potassium, and more recently acetazolamide. Our studies involve improving diagnostic methods, seeking the causes and analysing the pathogenic mechanisms, and improving methods of treatment.

In the hypokalemic form of PP, the treatment we introduced, long-term acetazolamide, has continued to be the best prophylactic agent both for preventing attacks and improving inter-attack weakness. It is now in the textbooks as such. Two of our patients have been treated successfully for more than 10 years. Renal calculi in one of 26 patients, possibly but not definitely related to the acetazolamide, have been the only suspected side-effect. Since muscle does not contain carbonic anhydrase, the mechanism of acetazolamide benefit in hypokalemic PP remains unknown -- we are investigating it.

Proposed Course of Project: To explore in more detail, with patients and animals, the mechanism of action of acetazolamide prophylaxis in hypokalemic periodic paralysis and the pathogenesis of the disease itself. To seek even better therapeutic agents.

Publications: None

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Myasthenia Gravis (MG)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Medical Neurology Branch

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INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20014

TOTAL MANYEARS:

3.0

PROFESSIONAL:

1.5

OTHER:

1.5

SUMMARY OF WORK (200 words or less - underline keywords)

To apply clinical, immunologic, histochemical, pharmacologic, electrophysiologic, autoradiographic, radionuclide-scanning, tissue-culture and electronmicroscopic techniques to investigate the etiology and pathogenesis of myasthenia gravis. To seek new or improved methods of treatment.

Project Description:

Objectives: To apply clinical, immunologic, histochemical, pharmacologic, electrophysiologic, autoradiographic, radionuclide-scanning, tissue-culture and electronmicroscopic techniques to investigate the etiology and pathogenesis of myasthenia gravis. To seek new or improved methods of treatment.

Methods Employed: A variety of basic and clinical investigative techniques, as detailed below, were applied to patients with myasthenia gravis and other disorders of neuromuscular transmission, and to induced animal-models thereof.

Patient Material: Myasthenia gravis patients, and patients with other disorders of neuromuscular transmission, participated in the investigative studies and therapeutic trials. Sera, muscle, thymus and other tissue were obtained during diagnostic or therapeutic procedures from these Medical Neurology Branch patients.

Major Findings:

Myasthenia gravis (MG) is an acquired disorder affecting transmission at the neuromuscular junction, mainly in adults and older children. The primary cause is not known but the pathogenic mechanism is considered to be "autoimmune" or "dysimmune". Untreated patients are usually seriously handicapped and many die. Palliative treatment with anticholinesterases and anti-pathogenic treatment consisting of thymectomy, ACTH and, most recently, prednisone has helped considerably but much disability, some fatality, and drug side-effects persist. Our studies involve improving diagnostic methods, seeking the cause and analyzing the pathogenic mechanisms, and improving methods of treatment. We also seek fuller understanding of the basic biology and pathologic responses of the neuromuscular junction.

Last year we reported the identification of a newly recognized blocking factor, an IgG, in the sera of MG patients which blocks binding of alpha-bungarotoxin (α BT) to the human junctional acetylcholine receptor (AChR) at the normal neuromuscular junction (41% of MG patients) and extrajunctional AChR of denervated human fibers (72% of MG patients, including all who had a thymoma) -- the latter being the more sensitive assay -- presumably because the factor itself is binding to the AChR (with NIHL). Now we have reported that in a correlative study all MG patients having the IgG antimuscle antibody (first found by others) also had the blocking factor (although only half having the latter had the former) (the only discordant finding was in one non-myasthenic myositis patient with thymoma who had antimuscle antibody but no detectable blocking factor) -- we suggest these may be the same antibody or, if different, apparently virtually always co-produced. The junctional localization of the blocking factor puts it in the correct position to impair neuromuscular transmission and cause the weakness of MG. Although we think this is likely, the factor has not been shown to produce weakness in any test system. We have not been able to induce weakness of newborn mice by injecting

IgG blocking-factor-positive MG sera during pregnancy or into the newborns. Blocking-factor-positive MG sera were shown to block the binding of α BT to the diffuse extrajunctional receptors of aneurally cultured human, rat, and chick muscle fibers (with NYU).

Since we have shown by electromyographic-immunohistochemistry that α BT binds, and presumably localizes AChR, at the neuromuscular junction both to the crests of the post-synaptic muscle membrane folds and to a lesser extent to the pre-synaptic axonal membrane, the blocking factor, if indeed pathogenic, would act both post- and pre-synaptically. Data from other types of studies by others support a pre- as well as a post-synaptic locus of AChR in normal neuromuscular junctions. On the basis of the blocking factor, we have revised our multistep hypothesis of the pathogenesis of MG and in doing so realized its striking analogy to the scenario of Verdi's Rigoletto. We therefore term the presumably-detrimental blocking factor a "Sparafucile" molecule after Verdi's hired assassin who without malice killed the unintended victim, in fact, killed the beloved daughter, Gilda (the AChR receptor in MG) of Rigoletto, who planned the assassination to be of the Duke of Mantua. It is evident from further analysis of the scenario that the answer to MG will only come with the identification and treatment or prevention of Count Monterone's curse (? an environmental factor, ?? viral).

We are investigating which cells, presumably B-lymphocytes, make the blocking factor in MG patients, and why, and how its production or presumed detrimental action can be prevented.

That the thymus is involved in many cases of MG is well known, but its role is not. Our counts of T- and B-lymphocytes in fresh thymus removed from MG patients during therapeutic thymectomy have not confirmed an increased percentage of the latter as reported by others. Mixed lymphocyte reactions with MG thymus are being analysed by light- and electronmicroscopy. Our EM of MG thymuses has thus far failed to reveal a virus, as have tissue culture studies. The multifactorial nature of the dysimmune phenomena in MG was reemphasized by the finding of anti-native-DNA antibody in the sera of a significant number of patients (with NIAMDD).

Working with the induced autoimmune model (rabbits injected with electric-fish AChR), originated by others, of experimental allergic MG (EAMG) (with Cornell) we demonstrated: (a) binding of that rabbit sera to human neuromuscular junctions but not to extrajunctional receptor at light-microscopic resolutions, (b) binding of it by electronmicroscopic resolution to the plasmalemma of cultured human, rat and chick fibers (with NYU), (c) binding of that sera to the original antigen in a radioimmunoassay we developed (with IB), (d) similarities but also distinct differences of the model with human MG, indicating it is not a perfect model of the latter although it could still be a model-in-principle, (e) toxicity of EAMG sera to reinnervated muscle in tissue culture which did not exceed the toxicity of normal rabbit sera (surprisingly toxic).

Confirmed and adopted by most other physicians has been the treatment we introduced to MG, long-term high-single-dose alternate-day prednisone (LT-HSDAD-Pred). In our own series it continues to be extremely beneficial in the majority of cases, 37 of 40, and for as long as 10 years in a child and 6 years in an adult. Responding best are the older-onset patients, especially the older males. Our 3 non-responders were females in the menstruating age group. Interestingly, none of our responding patients has become absolved of his requirement for prednisone, even after a gradual tapering of the dose. For example, two patients have exacerbated 3 months after stopping a 5 mg q.o.d. dose (this is about the length of time taken for resumption of abnormal IgG synthesis as measured by others in another disease). We have recently modified the treatment slightly by giving, to patients not simultaneously taking anticholinesterase drugs, the single-dose 100 mg prednisone daily for the initial 2-4 weeks before converting to the alternate-day schedule, apparently resulting in more rapid improvement. Fragile patients are not taken off their anticholinesterases but in them prednisone is given in a gradually incrementing single-dose-daily schedule beginning with 10-20 mg. Regarding combining anticholinesterase and prednisone, we find that low doses of one can be combined with the other advantageously -- but that patients taking both drugs sometimes seem to have a more "brittle" myasthenia and must be watched carefully for intractable overdosage by the anticholinesterase. However, because neither anticholinesterase nor prednisone treatment is either curative or completely preventative, more details of the pathokinesis are needed (v.s.).

The effect of the HSDAD-prednisone treatment on lymphocytes was measured over a 48-hour cycle in a number of MG and other patients. At 6 hrs. after the 8:00 a.m. prednisone dose there is marked depression of T-lymphocyte counts and lymphocyte responses to T-lymphocyte mitogens with a lesser effect on B-lymphocytes and response to B-lymphocyte mitogens, and there was return of these measurable effects to normal by 24 hrs. after the dose -- yet clinically the prednisone has a cumulative effect over weeks and months.

Partial sternal-splitting continues to be our preferred approach to thymectomy (with NIHL) -- the mortality is essentially zero. The suprasternal approach for us is inadequate for satisfactory removal of all thymic tissue. We continue to demonstrate that "out-of-control" MG patients often can be remarkably improved by treating co-existent medical problems such as chronic respiratory infections, urinary tract infections, and anemias -- possibly co-existing, ameliorative problems must be sought in each MG patient by detailed general medical investigation.

The remarkable ancillary benefit that broad-aspect nursing can provide to an MG patient is repeatedly evidenced in our patients -- our multidimensional nursing care approach for myasthenics has been videotaped (with Nursing, CC) and made available for general distribution -- this undoubtedly will help improve the care and perhaps save the lives of some myasthenic patients with serious disease, especially in hospitals not frequently caring for such patients.

With i.v. edrophonium (Tensilon), contrary to its usually considered short action of 5-10 min., we have preliminarily reported the documentation by detailed clinical and electromyographic testing an improvement in strength and neuromuscular transmission lasting 1-2 hrs. in several MG patients. This has practical importance since repeated edrophonium tests are commonly used (overused), without careful vital capacity monitoring, for adjusting dosage of other anticholinesterases.

A new electrophysiologic type of defect of neuromuscular transmission has been found in a patient with a fatigue syndrome and chronic renal disease -- after a brief tetanus there is a diminished but broadened total-muscle action-potential which gradually recovers during rest or slow stimulation, and it is not ameliorated by edrophonium, guanidine, or Ca^{++} .

We have just completed setting up the electromyographic equipment to study "jitter" and "blocking" phenomena recently described by others -- it is important diagnostically and investigatively in MG patients.

Significance to Bio-Medical Research and the Program of the Institute: These findings present new information on the pathologic and pathogenic aspects of myasthenia gravis, and other defects of neuromuscular transmission, on treatment, and on corresponding animal-models.

Proposed Course of Project: To develop more fully the interlinked basic and clinical studies underway directed toward clarification of the pathogenesis and identification of the etiology, and toward elaboration of better means of treatment and prevention.

Publications:

Rosenbaum, R. B., Bender, A. N. and Engel, W. K.: Prolonged response to edrophonium in myasthenia gravis. Trans. Am. Neurol. Assoc. 100:233-235, 1975.

Engel, W. K.: Myasthenia gravis, corticosteroids, anticholinesterases. Ann. N. Y. Acad. Sci., 1975, in press.

Trotter, J. L., Ringel, S. P., Cook, J. D., Engel, W. K., Eldefrawi, M. E. and McFarlin, D. L.: Experimental autoimmune myasthenia gravis (EAMG): Morphological and immunological studies. Neurology, 1976, in press.

Gershwin, M. E., Glinski, W., Bender, A. N., Ringel, S. P., Steinberg, R. D. and Engel, W. K.: Autoantibodies in myasthenia gravis. Int. Arch. Allergy Appl. Immunol., 1976, in press.

Bender, A. N., Ringel, S. P., Engel, W. K., Vogel, Z. and Daniels, M. P.: Immunoperoxidase localization of alpha-bungarotoxin binding -- A new approach to identifying denervated skeletal muscle fibers in myasthenia gravis. Ann. N. Y. Acad. Sci., 1975, in press.

Ringel, S. P., Bender, A. N., Engel, W. K. and Smith, H. J.: Myasthenia gravis: Relationship between serum factor blocking acetylcholine receptors and anti-striated-muscle antibody. Lancet I:1388, 1975.

Bender, A. N., Ringel, S. P. and Engel, W. K.: The acetylcholine receptor in normal and pathologic states: Immunoperoxidase visualization of alpha-bungarotoxin binding at a light and electron-microscopic level. Neurology 26:477-483, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01191-12 MN
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) Immunological Abnormalities of Neurologic Diseases		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: W. King Engel, M.D., Chief, Medical Neurology Branch, NINCDS Jay D. Cook, M.D., Medical Officer, MN NINCDS John L. Trotter, M.D., Medical Officer, MN NINCDS OTHER: Adam N. Bender, M.D., Assistant Professor of Medicine, Mt. Sinai Hospital, New York, NY Steven P. Ringel, M.D., Chief of Neurology, University of Colorado Medical Center, Denver, CO Valerie Askanas, M.D., Ph.D., Research Assistant Professor, Institute for Rehabilitation Medicine, New York University, New York, NY M. E. Gershwin, M.D., Assistant Professor of Medicine, University of California Medical School, Davis, CA		
COOPERATING UNITS (if any) Mt. Sinai Hospital, New York, NY University of Colorado Medical Center, Denver, CO Institute for Rehabilitation Medicine, New York University, New York, NY University of California, Davis, CA		
LAB/BRANCH Medical Neurology Branch		
SECTION --		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20014		
TOTAL MANYEARS: 3.1	PROFESSIONAL: 1.5	OTHER: 1.6
SUMMARY OF WORK (200 words or less - underline keywords) For a more clear identification of the association of the basic studies covered by this project with the various clinical diseases to which they relate, all aspects of this project have been incorporated into our on-going projects, numbers - Z01 NS 01034-14 MN Z01 NS 01039-14 MN Z01 NS 01189-08 MN Z01 NS 01190-12 MN		

Z01 NS 01192-12 MN

PERIOD COVERED July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Electronmicroscopic Studies of Skeletal Muscle and Neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: W. King Engel, M.D., Chief, Medical Neurology Branch, NINCDS
Valerie Askanas, M.D., Ph.D., Research Assistant Professor, Institute
for Rehabilitation Medicine, New York University, New York, NY

OTHER: Adam N. Bender, M.D., Assistant Professor of Medicine, Mt. Sinai
Hospital, New York, NY
John W. Griffin, M.D., Assistant Professor of Neurology, Johns Hopkins
School of Medicine, Baltimore, MD
Steven P. Ringel, M.D., Chief of Neurology, University of Colorado
Medical Center, Denver, CO
Martin L. Fishman, M.D., Clinical Associate, Clinical Branch, NEI

COOPERATING UNITS (if any)

Institute for Rehabilitation Medicine, New York University, New York, NY
Mt. Sinai Hospital, New York, NY
Johns Hopkins School of Medicine, Baltimore, MD (see below)

LAB/BRANCH
Medical Neurology Branch

SECTION

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INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20014

TOTAL MANYEARS:

4.0

PROFESSIONAL:

1.5

OTHER:

2.5

SUMMARY OF WORK (200 words or less - underline keywords)

For a more clear identification of the association of the basic studies covered by this project with the various clinical diseases to which they relate, all aspects of this project have been incorporated into our on-going projects, numbers - Z01 NS 01034-14 MN
Z01 NS 01039-14 MN
Z01 NS 01189-08 MN
Z01 NS 01190-12 MN

Cooperating Units (Continued):

University of Colorado Medical Center, Denver, CO
Clinical Branch, NEI

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01193-12 MN

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Radioautography Applied to the Study of Neurologic Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: W. King Engel, M.D., Chief, Medical Neurology Branch, NINCDS
OTHER: John W. Griffin, M.D., Assistant Professor of Neurology, Johns Hopkins
School of Medicine, Baltimore, MD
Donald L. Price, M.D., Associate Professor of Neurology & Neuropathology,
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COOPERATING UNITS (if any)

Johns Hopkins School of Medicine, Baltimore, MD

LAB/BRANCH

Medical Neurology Branch

SECTION

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INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20014

TOTAL MANYEARS:

1.8

PROFESSIONAL:

1.2

OTHER:

0.6

SUMMARY OF WORK (200 words or less - underline keywords)

For a more clear identification of the association of the basic studies covered by this project with the various clinical diseases to which they relate, all aspects of this project have been incorporated into our on-going projects, numbers - Z01 NS 01034-14 MN
Z01 NS 01039-14 MN
Z01 NS 01189-08 MN
Z01 NS 01190-12 MN

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 01792-07 MN

PERIOD COVERED July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Electrophysiology Applied to the Study of Neurologic Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: W. King Engel, M.D., Chief, Medical Neurology Branch, NINCDS
Richard B. Rosenbaum, M.D., Resident in Neurology, University of California
Medical Center, San Francisco, CA
Tulio E. Bertorini, M.D., Visiting Associate, MN NINCDS
Roger A. Brumback, M.D., Clinical Associate, MN NINCDS

COOPERATING UNITS (if any)

Dept. of Neurology, University of California Medical Center, San Francisco, CA

LAB/BRANCH

Medical Neurology Branch

SECTION

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INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20014

TOTAL MANYEARS:

0.7

PROFESSIONAL:

0.7

OTHER:

0

SUMMARY OF WORK (200 words or less - underline keywords)

For a more clear identification of the association of the basic studies covered by this project with the various clinical diseases to which they relate, all aspects of this project have been incorporated into our on-going projects, numbers - Z01 NS 01034-14 MN
Z01 NS 01039-14 MN
Z01 NS 01189-08 MN
Z01 NS 01190-12 MN

ANNUAL REPORT

July 1, 1975 through June 30, 1976

Surgical Neurology Branch, IRP

National Institute of Neurological and Communicative Disorders and Stroke

A. K. Ommaya, M.D., F.R.C.S., Acting Chief

Summary of Studies in Surgical Neurology Branch

In spite of major administrative rearrangements and retrenchment of space and personnel from this branch, the primary orientation of our studies has been successfully revised. Surgically treatable diseases of the nervous system and the methods required to understand and manage such diseases are now the chief concern of our research projects. Studies are being pursued in the following six major areas using both clinical and experimental research protocols.

I. Brain Tumors

A. Experimental. Trials of active immunotherapy and adoptive immunotherapy in a murine glioma model have shown that significant increase of survival time occurred only with preimmunization and not with immunotherapy, using standard subcutaneous immunization techniques. Chemotherapy with CCNU in the same model was also successful in increasing survival time, and the effects of preimmunization and CCNU were additive. A model for the study of intracerebral immunologic reactions of the delayed hypersensitivity class (DH) in the subhuman primate was developed. Intracerebral DH reactions to PPD could be evoked safely in rhesus monkeys. This finding opens the avenue of using intratumoral DH reactions to induce regression in malignant gliomas similar to that shown in a variety of other neoplasms. We also demonstrated a significant correlation between in vivo intradermal PPD reactions and intracerebral PPD reactions, thus indicating that skin testing with DH-invoking agents could be used clinically to predict the occurrence and possible intensity of intracerebral DH reactions.

Currently, we are developing two new brain tumor model systems. First, in athymic nude mice we are implanting human glioma tissue cells (both subcutaneously and intracerebrally) in order to develop a reliable small animal model of a glioma more identical to the human case than our earlier murine glioma system. This will be used in basic studies on the immunobiology of gliomas as well as to test a variety of intratumoral DH-inducing agents and combination adjuvant therapies (e.g. cryotherapy and chemotherapy). Secondly, a large animal model is being developed in the subhuman primate to test early detection techniques, both neuroradiologic (e.g. computed tomography) and immunochemical.

B. Clinical Trials

A review of our experience in the management of 106 patients with cerebral gliomas has been completed. The data show that our combination

therapy of extensive surgical resection controlled by microsurgical technique plus radiotherapy, plus chemotherapy with oral CCNU and intratumoral 8-Azaguanine has resulted in mean survival times of 93 weeks for grade 4 malignant gliomas (glioblastoma multiforme). This pilot study is encouraging when compared to the mean survival times of less than 40 weeks for conventional therapy (surgery plus radiotherapy) in the historical data, and of 48 weeks for the best results of the NCI-supported multicenter Brain Tumor Study Groups' controlled study of the combination of surgery plus radiotherapy, plus CCNU chemotherapy only. A detailed analysis of our results is now in press. In order to test the apparent value of our technique of combined therapy, with special reference to our unique intratumoral method, a controlled study protocol is now in development which will compare the quality and quantity of survival in two sequentially randomized populations of malignant glioma patients, both receiving surgical implantation of an intratumoral device but only one group being given the intratumoral chemotherapy. A separate protocol to evaluate the adjuvant value of immunotherapy using intratumoral C. parvum to induce a DH reaction after completion of chemotherapy is also in planning.

Collaborative studies on nervous system neoplasia in which the branch plays a major role include a search for a viral etiology of human gliomas grown in tissue culture, using electromicroscopic and other techniques (with the Infectious Diseases Branch, NINCDS). A separate study demonstrating the value of intrathecal chemotherapy in meningeal leukemia has also been completed with the Pediatric Oncology Branch, NCI.

II. Stroke

This is now an active area for investigation by both the neuroradiologic and neurosurgical arms of our branch. Experimental studies in animal models as well as clinical trials in man are in progress.

A. Experimental

The transorbital MCA occlusion monkey stroke model of Garcia is being used to evaluate neuroradiologic techniques, including computed tomography for their value in understanding the pathophysiology of cerebral blood flow in strokes. Neuroradiologic techniques correlated with neurologic and neuropathologic observations are also being used in a study of the effect of venous obstruction on cerebral structure, function and blood flow in the monkey. To date the techniques required for these studies have been successfully developed.

The intracarotid silicone embolization primate stroke model of Molinari is being used to study alterations in neurotransmitter in the CSF at various stages during and after embolic strokes. Our preliminary data support the hypothesis that CSF GABA elevations could be indicative of "silent" infarctions, i.e. three-fold elevation of CSF GABA after embolization, with highest levels in animals having significant pathologic changes irrespective of the fact whether clinical symptoms were present or absent.

B. Clinical Trials

A randomized controlled clinical trial of microneurosurgical anastomosis between the superficial temporal artery and the middle cerebral artery (STA-MCA) for prevention of stroke in patients with transient ischemic attacks of the anterior cerebral circulation is now in progress. An outpatient stroke clinic has been organized and patient referrals to this study are increasing satisfactorily. This is a 3-year clinical trial, by which time it is hoped that statistically adequate numbers of patients will have been accrued to the two arms of the study.

A parallel clinical study of CSF neurotransmitter levels in TIA patients before and after entry into our clinical trial of STA-MCA surgery is also in progress. The goal is to determine if such CSF indices can provide useful diagnostic or prognostic data for cerebral ischemia.

III. Spinal Cord Disease

This area has long been an active field for close collaboration between the neuroradiologic and neurosurgical arms of our branch.

A. Experimental

Neuroradiologic methods, particularly angiography, are being used to develop correlations with neuropathologic changes after post-radiation spinal cord myelitis in monkeys. A similar approach is also being pursued to evaluate the blood-CNS barrier and its varying degree of damage by radiographic contrast media in a spinal cord model in monkeys. A threshold for post-angiographic paraplegia in this model has been defined for one type of contrast medium.

Spinal Cord Trauma is being investigated experimentally using a primate model which features cord trauma, produced with a programmable dynamic cord-impact device which may be applied without prior laminectomy. The resultant injuries are being studied with evoked potential and neuropathologic methods. Preliminary data suggest that it is the severity of the primary neural damage, and not the secondary vascular or neurotransmitter responses, that is the main determinant of the final outcome for reversible or irreversible paraplegia.

B. Clinical Trials

In a large series of patients the diagnostic value of spinal cord selective arteriography and radioisotope angiography have been well demonstrated. The former radiographic method provides key information to guide the neurosurgeon in cases of arteriovenous malformations, tumors, obstructive vascular disease, trauma and post-radiation damage of the spinal cord. The latter isotopic technique provides a convenient screening method for the more "high-flow" types of vascular lesions of the spinal cord. Angiography in six patients with thoracic disc herniations has provided valuable information to aid the neurosurgeon in the surgery of

such lesions. A study of CSF iodine content after angiography is also in progress as a basis for explaining post-angiographic paraplegia. In some patients this complication of angiography may be reversed by CSF replacement with normal saline, i.e. an attempt to wash out excess contrast media stagnating in the extracellular environment of spinal cord neurones.

A neurosurgical study of arteriovenous malformations of the spinal cord has now been completed and data in 100 cases is undergoing analysis. This is the largest personal series of surgically treated cases with this lesion in the world and the results of this study will be published in a monograph. Detailed neurosurgical techniques for management of all of the many variations of this lesion will be thus made widely available.

IV. Head Injury

This continues to be a major focus of our work although the constraints of the Clinical Center preclude the development of significant in-house clinical research in this area.

A. Experimental

A hypothesis which reconciles the paralytic and amnesic phenomena of cerebral concussion has been developed and tested by our experimental model in monkeys. Satisfactory correlations of clinical and experimental data predicted by our hypothesis have been shown. Current head injury research at such NINCDS Head Injury Research Centers as the University of Pennsylvania have been directly modeled on our concepts and methods. The recent pathologic data on fatal head injuries in man, reported by Dr. Hume Adams of Glasgow, have also served to strengthen our hypothesis which provides for the first time testable concepts for the mechanism of traumatic unconsciousness as well as other sequelae of head injury.

Current experimental investigations are serving to validate a comprehensive finite element mathematical model of the head-neck system which when complete will serve to predict tolerances and guide engineering design for head protection systems nationally as well as internationally.

The pathophysiology of head injury is being studied in our model by a combination of two non-invasive methods which we have pioneered in head injury research; namely, analysis of averaged evoked responses and the use of computed tomography. These data will be evaluated against morphologic and metabolic data obtained using our unique Ois facility. In addition, we are re-examining the problem of brain edema in head injury, a subject hitherto somewhat confused by contributions based on models not duplicating actual head injury, e.g. the cryogenic and cytotoxic models. These studies will form the prelude to experimental testing of new therapeutic techniques aimed at blocking those effects of the secondary responses of trauma which inhibit or retard optimal post-traumatic neural reintegration.

B. Clinical Trials

A clinical study of computed tomography in head injuries is being carried out in collaboration with the Departments of Neurosurgery and Radiology of the George Washington University Medical School. This is a retrospective analysis of clinical, CT and pathologic data (where available). The necessity of CT in head injury management and also in the possible prediction of final outcome are the main aspects of this study.

V. The Cerebrospinal Fluid

A. Experimental

An experimental model for chronic ventricular and lumbar CSF fluid sampling and drug injections has been developed in monkeys with the Pediatric Oncology Branch. The pharmacokinetics and neurotoxicity of chronic intrathecal methotrexate and 8-Azaguanine therapy are being investigated. This model has been shown to be ideal for chronic physiologic, chemotherapeutic and neurotoxicity studies wherein the role of the CSF and its pathways have to be evaluated. In a separate neuroradiologic study the spinal flow of CSF has been demonstrated and correlated with clinical observations.

B. Clinical Trials

The techniques of isotope ventriculography and cisternography developed in this branch have proven to be of considerable clinical use to the neurosurgeon treating hydrocephalus, both infantile and adult. Work in this year has provided additional potential diagnostic uses in the management of porencephaly and hemispheric tumors. The circulation and particularly the spinal descent of CSF followed by such techniques has also been clearly described for the first time in man.

Neurochemical studies of the CSF in a variety of neurologic and psychiatric disorders have also been conducted in collaboration with investigators in the NIMH. Plasma and CSF Norepinephrine (NE) levels in patients undergoing caudate nucleus stimulation during surgical thalamotomy for involuntary movements were measured and significant reductions in CSF NE levels were found, contrary to earlier reports of NE increases in CSF. CSF NE was also found to be significantly decreased in patients with caudate atrophy (Huntington's chorea) as compared to age and sex-matched controls, suggesting a neurochemical basis for the autonomic dysfunction in Huntington's chorea patients.

CSF NE levels were also found to change after chronic cerebellar stimulation for epilepsy. Thus NE levels rose significantly after 16 hours of bilateral cerebellar stimulation. This alteration in NE may reflect changes in NE metabolism in epilepsy and may relate to the clinically noted suppression of seizures by cerebellar stimulation. Thus animal studies have shown that intraventricularly injected NE can abort experimental convulsions.

VI. Technical Developments in Neuroradiology and Neurosurgery

A. Computed Tomography

The most important technical advance in diagnostic medicine since the discovery of X-rays is undoubtedly the development of computed tomography (CAT). This technique is now a major tool of research in our branch and is the main area of research for the Section on Neuroradiology. Studies on leukoencephalopathies, Huntington's disease, post-radiation necrosis, and atypical teratomas have been pursued by this section. Scans carried out before and after positional change of head or eyes have provided added information. The technique of computer-assisted myelography using metrizamide has been introduced. A new type of CAT using protons which can detect differences in physical properties of material at the 0.1% level (as compared to the 0.5% level of current CAT devices) is being planned.

CT techniques are also being used in the serial evaluation of patients with head injury. Experimental studies of serial changes in the attenuation coefficients of the brain after head injury correlated with physiopathologic data are being planned.

B. A Neurosurgical Videomicroscope System

Microneurosurgery is probably the single most significant advance in neurosurgical technique since the improvement in anesthetic techniques facilitated operating on the brain and spinal cord. The operative mortality and morbidity of the neurosurgeon using the microscope for tumors, malformations and aneurysms has markedly decreased. Limiting factors in the more widespread use of and general acceptance of the microscope in neurosurgery are related to the generally poor human factors engineering in the operator-microscope-patient system. A careful study of these factors has resulted in a new concept for microneurosurgery embodied in the videomicroscope system, a purchase order for which has been submitted. It is believed that the purchase and use of this system will provide a unique national model for training neurosurgeons to adopt microneurosurgical procedures in all their major operations. It will certainly enhance our therapeutically directed research on brain tumors and strokes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 00907-15 SN																												
PERIOD COVERED July 1, 1975 to June 30, 1976																														
TITLE OF PROJECT (80 characters or less) Head Injury (Previous Title: Trauma to the Nervous System)																														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																														
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">A. K. Ommaya</td> <td style="width: 40%;">Actg. Chief, Surg. Neurol. Br.</td> <td style="width: 20%;">SN NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>J. Wood</td> <td>Medical Officer, PHS</td> <td>SN NINCDS</td> </tr> <tr> <td></td> <td>N. Gunby</td> <td>Neurosurgeon</td> <td>NR AFRI</td> </tr> <tr> <td></td> <td>W. Alter</td> <td>Physiologist</td> <td>NR AFRI</td> </tr> <tr> <td></td> <td>D. Carpenter</td> <td>Chairman, Dept. Neurobiology</td> <td>NR AFRI</td> </tr> <tr> <td></td> <td>L. Thibault</td> <td>Mechanical Engineer</td> <td>BE DRS</td> </tr> <tr> <td></td> <td>D. O. Davis</td> <td>Assoc. Chairman, Dept. Radiol.</td> <td>R GWU</td> </tr> </table>			PI:	A. K. Ommaya	Actg. Chief, Surg. Neurol. Br.	SN NINCDS	OTHER:	J. Wood	Medical Officer, PHS	SN NINCDS		N. Gunby	Neurosurgeon	NR AFRI		W. Alter	Physiologist	NR AFRI		D. Carpenter	Chairman, Dept. Neurobiology	NR AFRI		L. Thibault	Mechanical Engineer	BE DRS		D. O. Davis	Assoc. Chairman, Dept. Radiol.	R GWU
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COOPERATING UNITS (if any) Biomedical Engineering & Instrumentation Branch, Division of Research Services, NIH; Dept. of Neurobiology, Armed Forces Radiobiology Research Institute; Dept. of Radiology, George Washington Univ. Med. Ctr.																														
LAB/BRANCH Surgical Neurology Branch																														
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																												
6.5	3.5	3.0																												
SUMMARY OF WORK (200 words or less - underline keywords) Our work in experimental head injuries is along two major avenues using two experimental models. The first model is that of inertial loading in a subhuman primate with which we are validating a mathematical model which will be used for developing improved standards for head injury protection and prevention under the auspices of the Department of Transportation. This model will also be used to study the physiopathology of head injury, with particular reference to changes in <u>evoked potentials</u> , and in the serial computerized tomogram of the head as well as observations on the <u>cerebral blood flow</u> and metabolism of oxygen using oxygen ₁₅ . The second model consists of a series of studies on isolated neural tissues in which we are correlating the primarily neural effects of the trauma independent of the vascular system. This study correlates the mechanical loading with the cellular and membrane responses initially using isolated nerve axons in which the neural mechanism of reversible and irreversible trauma will be analyzed.																														

Project Description:

Objectives:

1. To understand the mechanisms of mechanical trauma to the nervous system and its responses to grades of such trauma.
2. To design rational methods for protection, prevention, diagnosis, prognosis and treatment of trauma to the brain and spinal cord.

Methods:

1. Utilizing programmable head accelerating devices, subhuman primates are subjected to controlled head accelerations to obtain reproducible injury at three levels: subconcussive, concussive with no sequelae and concussive with sequelae.
2. Utilizing O_{15} a study of cerebral blood flow and metabolism at varying levels of brain injury in our primate model is being developed. This study will compare the flow and metabolic changes with alterations in neurophysiologic functions as measured by somatosensory, visual and auditory evoked potentials. Correlation of these data with behavioral and pathologic data will enable a clear analysis of the primary and secondary effects of neural trauma.
3. Utilizing computed tomography, the serial noninvasive evaluation of head injury in our primate model will be carried out. These data will be correlated with similar data from head injured patients.
4. A study of the dynamics of brain swelling and brain edema in our head injury model using neurochemical and physiologic techniques has also been initiated.

Major Findings:

Based on the experimental work of the past 5 years a hypothesis for cerebral concussion has been developed and published. This hypothesis is able to explain for the first time both the paralytic and amnesic phenomena of cerebral concussion and predicts the possible mechanism for post-traumatic sequelae. Data produced from clinical observations and from our experimental model in the monkey have not been able to refute this hypothesis to date. The experimental methodology and research design leading to our current hypothesis have proven to be very successful and have been copied by the Head Injury Center at the University of Pennsylvania. Our hypothesis also predicted certain pathological findings for head injury in man which have recently been confirmed by Dr. Hume Adams in a report on fatal head injuries in Glasgow.

Our hypothesis has also served as a predictor for the finding of subdural hematomas in battered children where no evidence of direct head impact has been found. Pediatricians treating this condition have accepted the mechanism which we have proposed; namely, indirect accelerative effect on

the head produced by shaking the child, which thus results in large subdural hematomas without any evidence of direct blows to the head. Our preliminary work with computed tomography for head injury research has established the basis for correlating linear attenuation coefficients in vitro with the data found in vivo in patients. This study is being done in collaboration with the George Washington University Medical School.

Significance to Biomedical Research and the Program of the Institute:

1. Our work is continuing to provide the standard criteria nationally and internationally for experimental studies in neural trauma research.
2. Correlation of neurophysiologic (evoked potential), physiologic (cerebral blood flow and metabolism) and pathologic data in our experimental models will enable us to separate the primary and secondary effects of neural trauma.
3. Validation of a mathematical model for head injury will enable significant improvements in head injury protection and prevention strategies.

Proposed Course:

1. Develop the techniques of evoked potentials and C.A.T. as clinically practical, serial, noninvasive methods for diagnosis and prognosis of neural trauma severity. C.A.T. will be used to measure volumetric and blood brain barrier changes in the brain, both in the animal model and in patients after head injury.
2. Develop the method of cerebral compliance measurement as a prognostic index for brain survival in neural trauma.
3. Test new therapies aimed at blocking secondary responses to trauma and accelerating neural reintegration after injury.

Publications:

Ommaya, A.K. and Gennarelli, T.A.: Experimental Head Injury. In Vinken, P.J. and Bruyn, G.W. (Eds.): Handbook of Clinical Neurology. Injuries of the Brain and Skull. New York, American Elsevier Publishing Co., 1975, vol. 23, chapt. 4, pp. 67-90.

Ommaya, A.K.: Spinal Fluid Fistulae. In Keener, E.B. (Ed.): Clinical Neurosurgery. Baltimore, Maryland, Williams and Wilkins, 1976, chapt. 27. In press.

Ommaya, A.K.: Surgical management of head injuries in athletes. The Physician and Sportsmedicine 4: 56-63, 1976.

7-10-1914
P. 10-11-12

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01025-14 SN						
PERIOD COVERED <div style="text-align: center;">July 1, 1975 to June 30, 1976</div>								
TITLE OF PROJECT (80 characters or less) <div style="text-align: center;">Tumors of the Nervous System</div>								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: A. K. Ommaya</td> <td style="width: 33%;">Actg. Chief, Surg. Neurol.</td> <td style="width: 33%;">SN NINCDS</td> </tr> <tr> <td>OTHER: D. P. Houchens</td> <td>Senior Staff Fellow</td> <td>LEC NCI</td> </tr> </table>			PI: A. K. Ommaya	Actg. Chief, Surg. Neurol.	SN NINCDS	OTHER: D. P. Houchens	Senior Staff Fellow	LEC NCI
PI: A. K. Ommaya	Actg. Chief, Surg. Neurol.	SN NINCDS						
OTHER: D. P. Houchens	Senior Staff Fellow	LEC NCI						
COOPERATING UNITS (if any) <div style="text-align: center;">Laboratory of Experimental Chemotherapy, Division of Cancer Treatment, Drug Research & Development, National Cancer Institute</div>								
LAB/BRANCH <div style="text-align: center;">Surgical Neurology Branch</div>								
SECTION								
INSTITUTE AND LOCATION <div style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</div>								
TOTAL MANYEARS: <div style="text-align: center;">3.0</div>	PROFESSIONAL: <div style="text-align: center;">2.0</div>	OTHER: <div style="text-align: center;">1.0</div>						
SUMMARY OF WORK (200 words or less - underline keywords) <p>A controlled clinical study of the effect of <u>intratumoral chemotherapy</u> using 8-Azaguanine is being tested, comparing this method, given in combination with systemic CCNU after surgery and radiotherapy, to the effect of adjuvant <u>chemotherapy</u> with CCNU given alone. In a pilot study our methods have increased the mean survival time to approximately twice that reported by other centers using CCNU alone after surgery and radiotherapy. We are developing an experimental human <u>glioma</u> model in the <u>nude mouse</u> and this model will be used for chemotherapeutic and immunotherapeutic studies.</p>								

Project Description:

Objectives:

1. To develop surgical and immunochemotherapeutic methods for the treatment of human malignant gliomas and other "inoperable" tumors of the nervous system.
2. To evaluate immunologic parameters of patients undergoing therapy of brain tumors.
3. To develop animal models suitable for evaluation of immunotherapy and chemotherapy trials.
4. To evaluate the a) immunological identification of tumor-specific antigens; b) chemical makeup of tumor-associated antigens, initially assumed to be in glycoproteins isolated from tumor cell membranes; c) antigenicity of tumor-associated glycoproteins.
5. To improve the quality and quantity of survival of patients with malignant brain tumors.

Methods Employed:

1. Microneurosurgical techniques to enhance maximal reduction of tumor cell mass.
2. Patients with histologically verified glioblastoma multiforme and malignant astrocytomas (Grades III and IV) are selected. The extent of neurological deficit and intracranial mass anatomy is established clinically and neuroradiologically, including evaluation by computerized axial tomography before and after maximal tumor resection and treatment with radiotherapy.
3. Cerebrospinal fluid reservoirs and intratumoral cysts are inserted to allow evacuation of tumor-bed contents and for infusion of chemotherapeutic agents or agents to induce intratumoral delayed hypersensitivity reactions.
4. Patients were randomized into a prospective controlled study to evaluate combined chemotherapy with CCNU and 8-Azaquanine versus chemotherapy with CCNU alone. (Both groups receiving the intratumor cyst implant.)
5. The program of combined chemotherapy utilizes oral CCNU, 130 mg/sq. meter body surface, and intratumoral 8-Azaguanine, 100 mg. by infusion, the oral drug being given for 6 doses, one dose per 6-8 week period or until onset of liver or marrow disturbance. The intratumoral drug is given once a week for 6 weeks and then once a month for one year, and once a month indefinitely after that.
6. A murine glioma model has been developed which can reliably induce intracerebral tumors in mice and provide large numbers of cells for immunotherapy of that tumor. This animal model was also used to test varying combinations of immunotherapy, chemotherapy, radiotherapy and also to check the effect of splenectomy on tumor growth with and without therapy.
7. A model of human glioma in nude mice is being developed.

Major Findings:

The experimental model of a murine glioma has proven not to be adequate for further trials of immunotherapy, particularly for testing intratumoral immunochemotherapeutic techniques. Accordingly, a new model for the growth of human gliomas in nude mice is being developed which would allow large subcutaneous tumors to be used for this purpose. A primate model for intracerebral immunologic reactions has been developed and intracerebral delayed hypersensitive reactions have been studied. This finding opens the avenue for using intratumor DH reactions to induce regression in malignant gliomas similar to that shown in a variety of other neoplasms. A significant correlation between in vivo intradermal PPD reactions and intracerebral PPD reactions has been shown. This would indicate that skin testing with DH-invoking agents could be used clinically to predict the occurrence and possible intensity of intracerebral DH reactions.

We have reviewed our experience in 106 patients with cerebral glioma. Our combination therapy with extensive surgical resection controlled by micro-surgical technique, followed by radiotherapy and chemotherapy with oral CCNU and intratumoral 8-Azaguanine, has resulted in mean survival times of 93 weeks for Grade 4 malignant gliomas.

This pilot study is encouraging when one compares our results with the mean survival time of less than 40 weeks for conventional therapy (surgery + radiotherapy) as available in historical data. The NCI multicenter Brain Tumor Study Group has conducted a controlled study of the effect of surgery plus chemotherapy with CCNU after radiotherapy, and the mean survival time of this study was only 48 weeks. Our current controlled study is designed to test the hypothesis that our method holds promise for survivals of longer duration with better quality.

Significance to Biomedical Research and the Program of the Institute:

1. Active or adoptive immunotherapy of gliomas by subcutaneous inoculation technique will not add significantly to glioma management at the present stage of our knowledge.
2. Advances in the understanding of glioma immunotherapy would be of value for the understanding of solid tumor behavior in general.

Proposed Course:

1. Our current controlled clinical protocol evaluates the effect of intratumoral chemotherapy with 8-Azaguanine given in combination with systemic CCNU versus CCNU alone. Chemotherapy in both is conducted after radical surgical resection using microsurgical control and radiotherapy, using conventional supervoltage technique.

2. If the model of the human glioma in the nude mouse proves to be successful, we will pursue a study of various agents to produce maximum reduction of the glioma using intratumoral and local techniques.
3. The significance of vascular factors in brain neoplasia will be correlated with the immunologic data.

Publications:

Ommaya, A.K.: Immunotherapy of Gliomas: A Review. In Thompson, R.A. and Green, J.R. (Eds.): Advances in Neurology. New York, Raven Press, 1976, vol. 15, pp. 337-359.

Spiegel, A.M., Di Chiro, G., Gorden, P., Ommaya, A.K., Kolins, J. and Pomeroy, T.C.: Computerized axial tomographic scan as an aid in the diagnosis and treatment of intracranial atypical teratomas. Ann. Intern. Med. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01047-14 SN
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Isotope-Ventriculography and Isotope-Cisternography		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	G. Di Chiro M. K. Hammock G. S. Johnston A. E. Jones M. V. Green T. H. Milhorat W. A. Bleyer	Head, Section on Neuroradiology Senior Staff Fellow Chief, Nuclear Medicine Dept. Assistant Chief Chief, Applied Physics Section Chairman, Dept. of Neurosurgery, Children's Hospital of DC Clinical Associate, Pediatric Oncology Branch
		SN NINCDS SN NINCDS NM CC NM CC NM CC PO NCI
COOPERATING UNITS (if any) Nuclear Medicine Dept., Diagnostic Radioisotope Section, CC, NIH; Dept. of Neurosurgery, Children's Hospital of DC; Pediatric Oncology Branch, NCI, NIH		
LAB/BRANCH Surgical Neurology Branch		
SECTION Section on Neuroradiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: .5	PROFESSIONAL: .5	OTHER: .0
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> <u>Isotope-ventriculography</u> and <u>isotope-cisternography</u> are diagnostic tools permitting the morphologic and dynamic study of the cerebrospinal fluid pathways more accurately than has ever been possible with any other diagnostic test. </p>		

Project Description:

Objectives: A gamma emitting isotope injected within the cerebrospinal fluid pathways will permit in subsequent head scans the pictorial outline of the ventricular system (isotope-ventriculography) and of the subarachnoid intracranial spaces (isotope-cisternography). Information about the anatomical status of the cerebrospinal fluid cavities, and, by multiple serial scans, of the normal and abnormal dynamics of the cerebrospinal fluid itself will be obtained. The spinal CSF spaces may also be evaluated.

Methods Employed: The radioisotope cisternography and ventriculography procedures are now well established.

Recently we have devoted particular attention to one aspect of the CSF flow, i.e., its descent to the spinal subarachnoid space. Experiments have been carried out in the Rhesus monkey after injection of radiopharmaceuticals within the ventricular system. The scintiphotographic data are appraised with the assistance of a computer. Digital analysis is performed using a small dedicated computer (HP 5407A Hewlett Packard Scintigraphic Data Analyzer), mated to the Anger camera. For this purpose the pin-hole collimator is positioned so that the entire lateral length of the cerebrospinal space is within the field of view of the Anger camera detector. Nine region of interest cursors are drawn: one over the cerebral convexity, and one to include the entire spinal subarachnoid space. Time-activity curves are obtained from each region of interest, simultaneously without moving the animal for the next three and one-half hours.

Preliminary experience has been gained in clinical material on the descent to the spinal subarachnoid space of the CSF. Many patients, all newborn infants, and all with abnormalities of the CSF circulation--the majority was made up of cases of myelomeningocele--have been subjected to the following procedure. A radiopharmaceutical has been injected into the lateral ventricles of the brain and the descent of the cerebral spinal fluid into the spinal canal has been studied with an Anger camera, and in selected cases with the help of a computer. When the computer has been used, total gathering of the data has been attained above the entire spine for a period of at least one hour following the intraventricular injection. A number of patients affected by meningeal leukemia, and in whom radioisotope ventriculography was carried out, were also followed up with spine scanning for the propose of gaining experience with the spinal CSF descent.

Major Findings: During the current fiscal year we have:

1) Gained additional experience with cisternography in cases of porencephaly.

2) Added to the number of cases of supratentorial and hemispheric gliomas studied by radioisotope cisternography. The characteristic pattern of lack of ascent of the radiopharmaceutical on the tumoral side has been confirmed.

3) Injected radiopharmaceuticals within the lateral ventricles of Rhesus monkeys with the intent of studying the spinal CSF flow. A pattern of early spinal subarachnoid descent has been noted. This is followed first by equilibration, and later by decrease of spinal radioactivity as well as concomitant augmentation of cerebral convexity subarachnoid activity. When the radiopharmaceutical is introduced via the cisterna magna, the observed downward spinal pattern is even more marked, whereas an injection into the anterior basal cisterns is followed by a prevalent ascending direction of the tagged albumin toward the convexity of the brain.

4) Injected radiopharmaceuticals within the lateral ventricles of human patients (newborns, children and adults) and followed the spinal descent of the radionuclide.

5) Gained additional experience with radionuclide ventriculography in patients with intracerebral neoplasm, meningeal infection and low pressure hydrocephalus. Important applications were assessment of CSF shunts, determining distribution of intrathecally administered drugs, evaluating functional status of hydrocephalus, and assessing the progression of neoplastic invasion of the ventricular system.

Significance to Bio-Medical Research and the Program of the Institute: Legions of authors are studying this remarkable fluid (CSF) which still remains uncomprehended since Cotugno first described it in 1764. In particular, we now have a diagnostic tool to gather information about the "terra incognita" which is represented by the basal and convexity subarachnoid pathways.

Last year's investigations should have practical implication for the diagnosis and followup of such conditions as porencephaly and hemispheric glioma.

The CSF spinal descent studies should enable us to determine what is the importance of the spinal CSF route of flow as an alternative pathway of re-sorption. The observations of the spinal descent pattern of the CSF have also heuristic significance in regard to a possible analysis of metabolites and drugs distribution through the CSF from the endocranial cavity to the spinal theca.

Proposed Course of Project: Further information about the normal and abnormal cerebrospinal fluid cavities, and the normal pathologic flow of the CSF will be gathered by the techniques of isotope-cisternography and isotope-ventriculography.

Publications: Di Chiro, G., Hammock, M.K. and Bleyer, W.A.: Spinal descent of cerebrospinal fluid in man. Neurology 26: 1-8, 1976.

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Radiographic and Radioisotopic Angiography of the Spinal Cord

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Di Chiro	Head, Section on Neuroradiology	SN NINCDS
OTHER:	J. L. Doppman	Chief, Diagnostic Radiology Dept.	DR CC
	J. R. Herdt	Deputy Chief	DR CC
	A. K. Ommaya	Acting Chief	SN NINCDS
	L. Wener	Chairman, Dept. of Radiology, Cafritz Memorial Hospital, Wash., DC	
	G. S. Johnston	Chief, Nuclear Medicine Dept.	NM CC
	A. E. Jones	Assistant Chief	NM CC
	M. M. Mishkin	Professor of Radiology, Hospital of the University of Pennsylvania, Phil., PA	
	E. L. Timins	Resident, Neurosurgery	GWU

COOPERATING UNITS (if any) Diagnostic Radiology Dept., CC, NIH; Dept. of Radiology, Cafritz Memorial Hosp., Wash., DC; Nuclear Medicine Dept., CC, NIH; Dept. of Radiology, Hosp. of the Univ. of Pennsylvania, Phil., PA; Medical Examiner's Office, City of Philadelphia, Department of Public Health, Phil., PA (cont'd).

LAB/BRANCH

Surgical Neurology Branch

next page

SECTION

Section on Neuroradiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

.1

PROFESSIONAL:

.1

OTHER:

.0

SUMMARY OF WORK (200 words or less - underline keywords)

Selective arteriography (radiographic) of the spinal cord is a diagnostic technique which has proven to be very informative in cases of arteriovenous malformation, tumor, obstructive vascular disease, trauma, and postradiation damage of the spinal cord.

Radioisotope angiography of the spinal cord offers distinct advantages as a screening method, and in certain types of intraspinal pathology may give information not available by any other diagnostic test.

Cooperating Units: Department of Neurosurgery, George Washington University (Continued) Medical School, Washington, D. C.

Project Description:

Objectives: The introduction of cerebral angiography (1927) has markedly increased our knowledge of the vascular pathology of the brain. The vascular pathology of the spinal cord, on the other hand, remains a largely unexplored area.

Since 1964 we have been carrying out angiographic studies of the spinal cord and developed this technique into a reliable diagnostic tool. Selective injection of the contrast medium has made the difference between an occasional demonstration, and the consistent visualization of the spinal cord vasculature.

The usefulness of selective arteriography in cases of spinal cord arteriovenous malformations is now well established. We are continuing to use this technique to: 1) Learn more about the pathophysiology of the spinal cord arteriovenous malformations so that a better treatment of these important and frequent lesions may be developed. 2) Evaluate how useful spinal cord angiography is in cases of spinal cord tumors. 3) Establish whether or not this technique can be of diagnostic value in the study of obstructive spinal cord vascular disease. 4) Assess the usefulness of this technique in intervertebral disc pathology. 5) Evaluate the diagnostic possibilities of this procedure in posttraumatic spinal cord injury with or without vertebral fractures. 6) Establish the value and limits of newly introduced radioisotopic angiography of the spinal cord. 7) Explore possible emergency therapeutic means which could be employed to treat and cure, or at least minimize the effects of the dreadful postangiographic cord complications. 8) Acquire new information regarding the fine vasculature of the human spinal cord, with particular emphasis on the intrinsic vessels (sulcal or central arteries and other perforating or penetrating branches). This goal is accomplished by post-mortem microangiographic techniques in cadavers of all age groups. We are paying particular attention to cords of aged adults.

Methods Employed: Selective arteriograms with modern catheter techniques are carried out in patients in whom spinal cord vascular or tumoral lesions are suspected. The subtraction technique is used to better visualize the injected vessels. In addition, in the last fiscal year we have gained considerable experience with the direct radiographic magnification angiograms.

For the technique of radioisotope angiography of the spinal cord a bolus of 15 mCi of ^{99m}Tc human serum albumin (1 to 2 ml) is injected in a left antecubital vein. Immediately afterwards, cinescintiphotographic or rapid flow Polaroid views of the various segments of the spine are obtained with an Anger scintillation camera. In the last fiscal year our scintiphotographic data have been significantly ameliorated by a computer assisted analysis and reconstruction of images, as well as by isometric contour computer display of the data.

For the post-mortem studies of the vessels of the human spinal cords, (aged adults) we have used our previously developed microangiographic techniques.

Based on the observation made elsewhere, that in two patients who died soon after aortography with spinal cord complications, the iodine content in the CSF was enormously increased, we are attempting an emergency therapeutic method consisting of flushing out the "iodine contaminated" CSF.

Major Findings: During the current fiscal year we have:

1) Studied patients with spinal cord pathology due to intervertebral disc disease. Particularly rewarding has been the information obtained in cases of herniation of the thoracic discs (six patients). Direct radiographic magnification has been especially useful in these cases. By spinal cord arteriography, we can reach a definite diagnosis of this condition together with an evaluation of the degree of impingement on the cord and its vessels by the protruding disc. This diagnosis is indispensable for a correct surgical therapy of this pathological condition: the exposure of the herniated thoracic disc should not be achieved through posterior laminectomy, but rather through a lateral retropleural or lateral transpleural approach.

2) We have concentrated our attention on post-mortem microangiographic evaluation of the aged human cord with a comparative appraisal of the intrinsic vasculature in the various segments (cervical, high- and mid-thoracic, and thoracolumbar).

3) In several patients who, in other hospital centers, developed paraplegia immediately after abdominal aortography, flushing out of the CSF and substitution with normal saline solution has been carried out, on an emergency basis, under our suggestion and guidance. This lavage has resulted in every instance in a rapid amelioration of the paraplegia. The improvement has been concomitant with a decrease of the CSF iodine content. For comparison purposes we have been gathering CSF samples during the various stages of different angiographic procedures, particularly selective arteriography of the spinal cord. In patients without neurological complications the postangiographic raise of the iodine content in the CSF is minimal.

Significance to Bio-Medical Research and the Program of the Institute: Radiographic and radioisotopic angiography of the spinal cord are increasing our understanding of the large group of conditions in which vascular lesions of the cord represent the basic pathologic element.

Proposed Course of Project: Post-mortem microangiography of the aged adults' cords should offer new insights on such conditions as obstructive vascular disease of the cord due to arteriosclerosis and cervical spondylosis, and possibly on degenerative and demyelinating cord diseases.

We are "watching" for possible further technical developments of the

technique of selective arteriography of the spinal cord. We have recently established the value of direct radiographic magnification, and we are considering initiating the use of angiotomography for a better visualization of the smaller vessels, possibly the intrinsic arteries and veins of the cord.

Improved x-ray vascular contrast media will also enhance the diagnostic possibilities of spinal cord angiography. We are following very closely the recent developments in the area of polymeric, ion-balanced and non-ionic iodinated x-rays contrast media.

We will dedicate much of our attention to technical improvements in the newly introduced radioisotope angiography of the spinal cord. This method, which we are extensively using as a screening and followup procedure, could become a more definitive and informative diagnostic examination. By increasing our resolution through a computer-assisted reconstruction and enhancement of the images, we should be able to extract a lot of diagnostic information from this simple and innocuous technique.

Publications: Di Chiro, G.: Recognition of radiculomedullary arteries. (Letter to the Editor) Brit. J. Radiol. (In Press).

Di Chiro, G.: Arteries of Spinal Cord: A chart to be used as a guide to avoid unintentional abusive angiographic-contrast-media injections into arteries supplying the spinal cord. Eastman Kodak Company, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER <div style="text-align: right; font-weight: bold;">Z01 NS 01654-09 SN</div>
PERIOD COVERED <div style="text-align: center;">July 1, 1975 to June 30, 1976</div>		
TITLE OF PROJECT (80 characters or less) <div style="text-align: center;">Experimental Spinal Cord Angiography</div>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	G. Di Chiro M. K. Hammock J. R. Herdt J. Fein G. J. D'Angio K. Earle E. L. Timins	Head, Section on Neuroradiology Senior Staff Fellow Deputy Chief, Diag. Radiology Dept. (formerly of Armed Forces Radiobiology Research Institute, Bethesda, MD) Chairman, Dept. of Radiation Therapy Memorial Sloan-Kettering Cancer Center, NY Chief, Neuropathology Branch Resident, Neurosurgery
		SN NINCDS SN NINCDS DR CC AFIP GWU
COOPERATING UNITS (if any) Diagnostic Radiology Dept., CC, NIH; Armed Forces Radiobiology Research Institute, Bethesda, MD; Dept. of Radiation Therapy, Memorial Sloan-Kettering Cancer Center, NY; Armed Forces Institute of Pathology, Wash., DC; Dept. of Neurosurgery, GWU Medical School, Wash., DC		
LAB/BRANCH <div style="text-align: center;">Surgical Neurology Branch</div>		
SECTION <div style="text-align: center;">Section on Neuroradiology</div>		
INSTITUTE AND LOCATION <div style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</div>		
TOTAL MANYEARS: <div style="text-align: center;">.2</div>	PROFESSIONAL: <div style="text-align: center;">.2</div>	OTHER: <div style="text-align: center;">.0</div>
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>Experimental <u>spinal cord angiography</u> in the Rhesus monkey is increasing our understanding of the blood supply of the spinal cord both in physiological and pathological conditions.</p>		

Project Description:

Objectives: The clinical value of the NIH developed technique of selective arteriography in the management of arteriovenous malformations and tumors (in particular hemangioblastomas) of the spinal cord is now well established.

In order to expand the clinical applications of arteriography of the spinal cord we are working with experimental angiographic and microangiographic models in primates.

Previously, we have concentrated our attention on the area of experimental obstructive vascular disease of the spinal cord in the Rhesus monkey. In the past fiscal year much of our experimental investigation has dealt with an iatrogenic pathological condition: Postradiation myelomalacia (myelitis).

In the area of postradiation myelitis we are particularly interested in establishing whether the basic pathological lesion of this dreadful complication is primarily neurogenic or vascular.

Methods Employed: Preradiation angiographic studies (selective technique) of the thoracolumbar segment of the spinal cord are carried out in young, healthy Rhesus monkeys. Soon after, selective irradiation of the thoracolumbar cord using the LINAC accelerator (A.F.R.R.I.) is initiated. Total dosage and modalities of delivery are chosen to approximate the radiation protocol which most often seems to cause myelomalacia in human patients.

At the end of the radiation, the monkeys are kept under careful observation for periods of many months. Neurological testing of the lower limbs is performed twice a week. If and when the monkeys show signs of developing or established paraplegia, repeat selective arteriography of the irradiated segment is carried out. Following this, the animals are perfused for microangiography of the spinal cord and then sacrificed. The cord is studied by gross observation, microangiography, routine histology and special myelin stains. Careful gross and histological analysis of the neighboring aortic segment, its branches and the pertinent radiculomedullary arteries is also carried out.

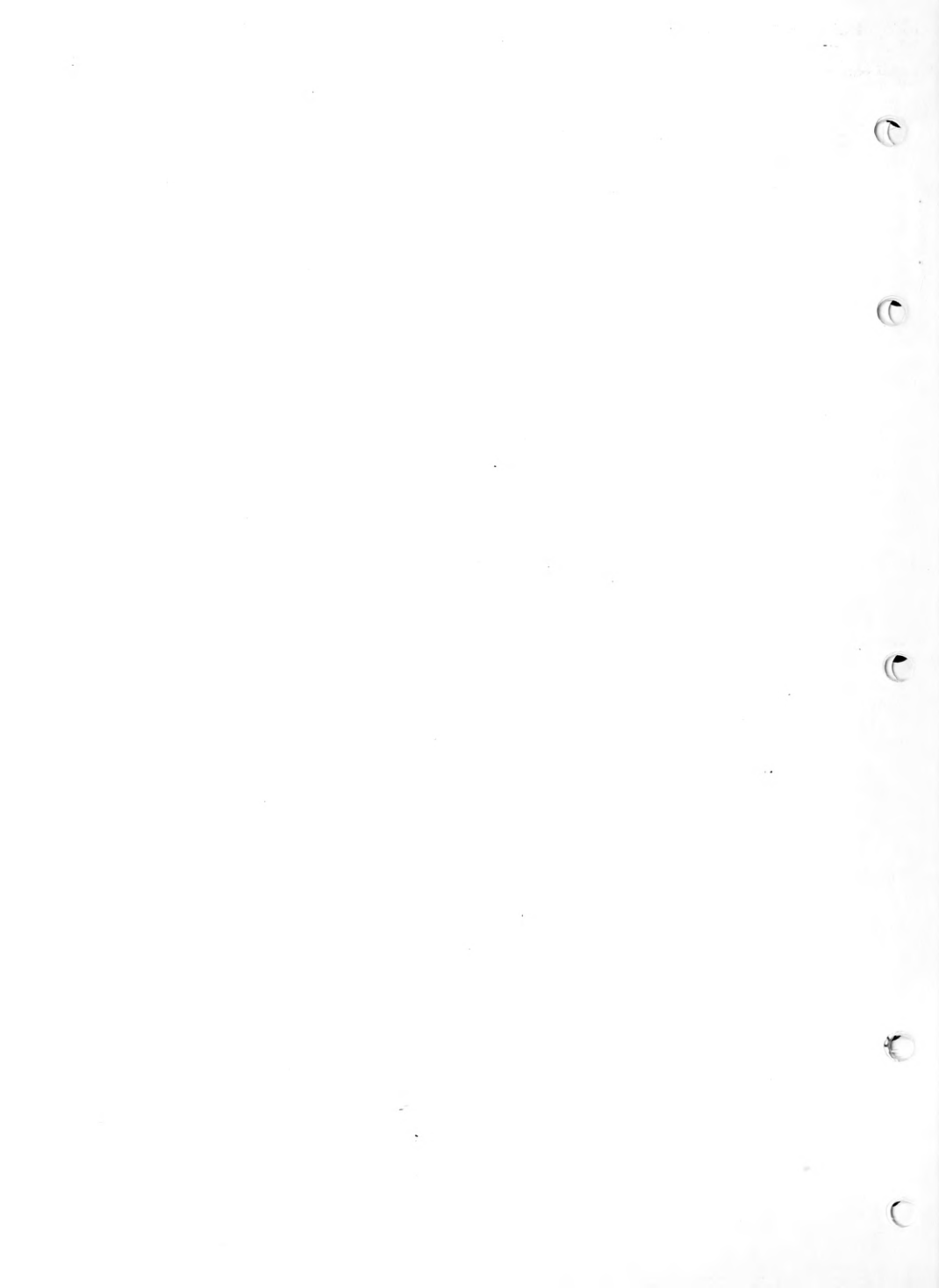
Major Findings: We are on the course of evaluating the pathological changes of the spinal cord from monkeys in which we successfully induced postradiation paraplegia (myelopathy).

Significance to Bio-Medical Research and the Program of the Institute: We should be able to shed some light on the pathogenesis of the postradiation myelitis. This is not a rare complication in human patients (over 500 cases have been reported in the literature).

Proposed Course of Project: Appraisal of the postradiation data which we have already collected as well as new data in other irradiated animals now under observation. We will attempt to study (by angiography and micro-

angiography) human patients (or human specimens) with postradiation spinal cord damage.

Publications: None



Project Description:

Objectives: "Stroke" is one of the pathological conditions which recently has been a target attacked from many different sides. For instance, in the last ten years, a great amount of research has been dedicated to the angiographic evaluation of the cerebrovascular obstructive disease. The major emphasis, however, has been put on the arteriographic findings. A perusal of the literature clearly shows that only scattered observations have been made regarding the phlebographic changes. The neuropathological studies (Hahn, Loeb, Zülch), on the other hand, have pointed out the fact that the obstructive venous pathology, although not as frequent as its arterial counterpart, represents an important entity.

We intend to explore the angiographic changes occurring in the cerebral vessels after experimental obstruction of the various brain venous channels (veins and dural sinuses) in a primate experimental model. We are particularly interested in establishing, on the basis of these experiments, which venous occlusions can be well tolerated and which are, instead, incompatible with good function. We will try to appraise, again angiographically, what types of compensatory mechanisms take place after the venous ligations. Do new venous draining pathways open, and if so, which and how efficient are these reserve discharge routes? The angiographic work will be carried out in close connection with an analysis of the clinical status of the operated animals.

The knowledge acquired in our experimental models will be extended to human material.

Methods Employed: Catheter arteriographic studies in the monkey (*Macaca mulatta*) are performed before and after ligation of one or several of the large endocranial venous channels.

Together with the post surgical angiographic evaluation, an analysis of the clinical condition of the monkeys is carried out. After various periods of time following the venous ligations, the animals are finally sacrificed in order to carry out microangiographic and gross and microscopic neuropathologic studies.

We are planning microangiographic studies of brains (supplied by the AFIP) of patients who have died of possible cerebral thrombophlebitis.

Major Findings: We have acquired a significant degree of experience in the evaluation of the monkey cerebral angiography. We have been impressed by the remarkable similarity of the major, and even medium size vessels (arteries, veins, dural sinuses) in the Rhesus monkey and man. We have good evidence, therefore, that the primate model will be applicable to the goals of our research project.

We have carried out in a group of Rhesus monkeys, ligation of the superior longitudinal sinus in its anterior, middle or posterior third.

Cerebral angiographic studies have been performed before and at various time intervals after the ligation. We are now in the process of evaluating our angiographic, surgical, gross and light microscopic anatomo-pathologic findings in the operated monkeys.

Significance to Bio-Medical Research and the Program of the Institute:
This project can increase our understanding of the pathophysiology of the cerebrovascular occlusive disease. Possibly, we will be able to recognize the separate specific conditions (mainly due to venous drainage impairment) in the large group of pathologic entities which are classified under the heading of "stroke".

Proposed Course of Project: We intend to continue our experimental investigation in monkeys.

Later on we intend to apply the acquired experimental knowledge to the human clinical experience.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER <div style="text-align: right; font-size: 1.2em;">Z01 NS 01866-06. SN</div>
PERIOD COVERED <div style="text-align: center; font-weight: bold;">July 1, 1975 to June 30, 1976</div>		
TITLE OF PROJECT (80 characters or less) <div style="text-align: center; font-weight: bold;">Studies on Cerebral Blood Flow by Radiographic and Radioisotopic Methods</div>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	G. Di Chiro M. K. Hammock A. Pongpatirojana P. C. Williams	Head, Section on Neuroradiology Senior Staff Fellow Staff Fellow Staff Fellow
OTHER:	N. D. Peters M. V. Green H. Agress, Jr. G. S. Johnston A. E. Jones S. L. Bacharach K. Earle	Clinical Associate Chief, Applied Physics Section Medical Research Analyst Chief, Nuclear Medicine Dept. Assistant Chief Physicist, Applied Physics Section Chief, Neuropathology Branch
		SN NINCDS SN NINCDS SN NINCDS SN NINCDS SN NINCDS NM CC LAS DCRT NM CC NM CC NM CC AFIP
COOPERATING UNITS (if any) Laboratory of Applied Studies, Division of Computer Research and Technology, NIH; Nuclear Medicine Department, CC, NIH; Armed Forces Institute of Pathology, Wash., DC		
LAB/BRANCH Surgical Neurology Branch		
SECTION Section on Neuroradiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: <div style="text-align: center;">.16</div>	PROFESSIONAL: <div style="text-align: center;">.16</div>	OTHER: <div style="text-align: center;">.0</div>
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> An experimental stroke model in the Rhesus monkey has been developed. The "stroked" animals are being evaluated by x-ray angiography, radionuclide scanning, radionuclide cerebral blood flow determination, radionuclide particles (macroaggregates) transit, fluorescein angiography, Laser-Doppler velocimetry, microangiography, and autoradiography. The CBF in the experimental infarcted brain is being modified by various physiological, chemical, pharmacological and surgical (revascularization) means. In a collateral experiment various parameters of the cerebral circulation are being studied after production of arterial spasm in the circle of Willis in monkeys. The spasm is caused by total blood -or fractions thereof- introduction within the subarachnoidal space (chiasmatic cistern). </p>		

Project Description:

Objectives: Although an enormous number of studies have been carried out on the cerebral blood flow (CBS), the actual behavior of the small cerebral vessels (arteries, capillaries, and veins) in physiologic and pathologic conditions is not yet clear. In fact, in recent years, new concepts--luxury perfusion, intracerebral steal, blue arteries and red veins, loss of autoregulation--have been introduced. These concepts have not brought about increased clarification, but rather additional confusion. We are convinced that morphological analysis of the vessels still remains a potential source of significant information in the area of normal and pathological CBS. We intend, therefore, to pursue the matter of extracting information regarding the circulatory conditions of the brain from radiographic observations, substantiated in selected instances by radioisotopic methods and possibly other diagnostic and research techniques.

Methods Employed: Radiographic and radioisotopic observations of the cerebral circulation represent the main tools to be used in this study.

An experimental stroke model in Rhesus monkeys has been developed. The middle cerebral artery is occluded at its origin through the superior orbital fissure after the removal of the eyeball. This technique (Garcia) offers various advantages. Particularly important is the fact that the produced infarction is consistent in its anatomical location (medial-inferior district of the ipsilateral basal ganglia) and its extent. Also, because no craniectomy is needed, subsequent studies with radioisotopic scanning are easily interpretable.

Two additional technical modalities are used for the evaluation of our "poststroke" experimental animals. One of these modalities is original and consists of the indwelling carotid catheter. This permits us to carry out a variety of injections and blood sample collections at serial intervals during long postoperative periods (weeks and even months). The second modality is the application of standard lucite calvaria for direct observation of the cerebral cortex.

After production of the anemic infarction our multi-pronged evaluation includes:

- A) Periodic neurologic testing.
- B) Periodic conventional static radionuclide brain scanning.
- C) Periodic magnification internal carotid angiography (an indwelling catheter is positioned in the internal carotid artery of some of the stroked monkeys).
- D) Periodic radioisotopic angiographic studies (Tc pertechnetate) and periodic ¹³³Xe washout studies (through indwelling internal carotid catheter).

All the radionuclide flow data are gathered with an Anger camera and stored on tape. Subsequently, the data on tape are analyzed by a computer-assisted program. Multiple cursors are drawn in the central part and at the periphery of the infarcted area. The purpose of this approach is to evaluate the centripetal and centrifugal blood flow in the infarcted area at various stages of development of the infarction. The data should be of assistance in the evaluation of the circulation parameters in the initiating, evolving, complete, and resolving stroke. The centripetal and centrifugal flow is evaluated second by second after the intracarotid injection of the radiopharmaceutical.

E) Acute polarographic deep electrodes are used in some of these animals for recording oxygen availability.

F) At various intervals after the creation of the stroke, the animals are sacrificed by a "sudden death" technique and immediately afterwards perfused with a special micropaque solution for post-mortem microangiographic studies.

G) The "sudden death" is accomplished under various attempts at modifying the pathophysiology of the CSF: hyperventilation, hypercarbia, anoxia, hyper- and hypo-tension, use of vasodilators.

H) In a few monkeys perfusion with the radioiodinated albumin macro-aggregates for scanning appraisal of the infarcted hemisphere has also been carried out.

I) In selected monkeys the changes of the superficial vasculature are followed up by means of fluorescein angiography of the exposed cortical surface.

J) In selected monkeys Laser-Doppler velocimetry is used to evaluate superficial (cortical) CBF in the various size vessels. This is accomplished with a preparation which includes the lucite calvaria and the indwelling carotid catheter.

K) A day-by-day histological analysis of the anemic infarction is underway. Stroked monkeys are sacrificed for this purpose, one at each sequential day, after the production of the stroke.

L) We plan to study the infarcted monkeys by Computer Assisted Tomography (CAT) as soon as a device for this technique (EMI-Scanner) will be available to us.

M) We are getting ready to start "revascularization" of our infarcted monkeys with an original method of by-pass procedure to the middle cerebral artery distal to the occlusion. The "revascularized" monkeys will be subjected to all the above multi-pronged testing.

In a collateral experiment various parameters of the cerebral circulation are being studied after production of arterial spasm in the circle of Willis

in monkeys. The spasm is caused by total blood, or fractions thereof, introduction within the subarachnoidal space (chiasmatic cistern).

Major Findings: We are very pleased with the experimental stroke model which we have developed. The possibilities of investigation with this model are practically unlimited. We have started evaluating the many data available to us from the first group of infarcted monkeys.

1) The stroke model used gives a consistent area of infarction in deep cerebral layers with a minimal amount of brain exposure.

2) Microangiograms prepared in the manner described can fix at a point in time the dynamic changes taking place in the microvasculature.

3) The method allows us to duplicate in deeper structures some of the observations of others on the reaction of surface vessels.

4) We can demonstrate changes in vessel morphology following PaCO_2 changes both in normal and infarcted monkeys.

5) Both hypo- and hyper-capnia decrease the perfusion of zones of ischemia during the acute phase. Hypocapnia accelerates the rate of ischemia to a greater extent than hypercapnia.

6) In completed stroke at the five day interval, hyperventilation hypocapnia may increase perfusion of nonischemic portions of the brain as compared to normocapnic and hypercapnic animals.

7) By polarographic depth electrodes determination, a reversal of the normal O_2a response in the ischemic brain to either low or high PaCO_2 levels has been observed. This would suggest that attempts at manipulation of cerebral blood flow to ischemic or infarcted brain using hypo- or hyper-capnia would not be consistently successful due to the variable response obtained.

8) A correlative study between sequential radionuclide brain scanning and time-lapse microangiograms has been completed. In the majority of animals brain scintigraphy becomes positive by two weeks to regress toward negative by four to six weeks after the ligation of the middle cerebral artery. The increased radioisotope uptake in the affected area is clearly related to neovascularization around the area of infarct as shown by the microangiograms. Decreased vascularity, peripheral gliosis and central cavity formation are the main factors determining the diminution of the radionuclide penetration in the involved area at later stages.

Significance to Bio-Medical Research and the Program of the Institute: We are hopeful that the computer analysis of our radioisotope angiography data in and around the infarcted area will be informative. In particular the determination of the centripetal and centrifugal blood flow in the area of the lesion should enable us to draw some conclusion on the mechanisms of resolution of the infarction and perhaps a better understanding of the important

phenomenon of the luxury perfusion.

We are confident that with our stroke model we will be able to contribute both in the basic area of CBF pathophysiology of the infarcted brain, as well as in the practical management of the patients with stroke. Perhaps we will shed some light on such fundamental questions as: a) if and when we should hyperventilate patients with stroke; b) when, if ever, induced hypercarbia may be useful in these patients; c) what is the optimal level at which the blood pressure should be maintained during the various stages of stroke; d) if, when, and which vasodilators should be used.

We have hopes that our "revascularization" approach may prove to be efficacious, practical, and applicable to human patients.

The arterial spasm production after subarachnoidal injection of blood into the chiasmatic cistern is an excellent model simulating the important problem of human arterial spasm following subarachnoidal hemorrhage. This event generally follows rupture of arterial and arteriovenous aneurysms of the brain.

Proposed Course of Project: This is a long-range research project. The evaluation of the cerebral blood flow both in experimental models and in clinical material is still in its infancy, but we are convinced that it will develop into a full discipline.

Publications: Peters, N.D. and Di Chiro, G.: A model for spasm of the anterior cerebral artery. Stroke (In Press).

Hammock, M.K., Di Chiro, G. and Pongpatirojana, A.: Technique for placement of experimental indwelling carotid artery catheter. J. Neurosurg. (In Press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 NS 02073-03 SN</div>
PERIOD COVERED <div style="text-align: center; font-weight: bold;">July 1, 1975 to June 30, 1976</div>		
TITLE OF PROJECT (80 characters or less) <div style="text-align: center; font-weight: bold;">Computer Assisted Tomography</div>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	G. Di Chiro R. A. Brooks D. B. Calne P. F. Teychenne J. L. Doppman J. R. Herdt M. Vermess G. S. Johnston A. E. Jones R. L. Webber R. N. Nagel N. E. Alexander R. S. Ledley H. K. Huang D. Schellinger	Head, Section on Neuroradiology Senior Staff Fellow Clinical Director Clinical Associate Chief, Diagnostic Radiology Dept. Deputy Chief Assistant Deputy Chief Chief, Nuclear Medicine Dept. Assistant Chief Chief Senior Staff Fellow Electronics Engineer Prof. of Radiology Assistant Prof. of Physiology & Biophysics Assistant Prof. of Radiology
		SN NINCDS SN NINCDS IRP NINCDS IRP NINCDS DR CC DR CC DR CC NM CC NM CC CIB NIDR CIB NIDR BEIB DRS GTU GTU (cont'd. next page)
COOPERATING UNITS (if any) Diagnostic Radiology Dept., CC, NIH; Nuclear Medicine Dept., CC, NIH; Clinical Investigations Branch, NIDR, NIH; Biomedical Engineering & Instrumentation Branch, DRS, NIH; Dept. of Radiology GTU Medical School, Wash. DC; Physics Dept., Tufts Univ., Medford, MA		
LAB/BRANCH <div style="text-align: center; font-weight: bold;">Surgical Neurology Branch</div>		
SECTION <div style="text-align: center; font-weight: bold;">Section on Neuroradiology</div>		
INSTITUTE AND LOCATION <div style="text-align: center; font-weight: bold;">NINCDS, NIH, Bethesda, Maryland 20014</div>		
TOTAL MANYEARS: <div style="text-align: center; font-weight: bold;">.18</div>	PROFESSIONAL: <div style="text-align: center; font-weight: bold;">.18</div>	OTHER: <div style="text-align: center; font-weight: bold;">.0</div>
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>Computer Assisted Tomography (CAT) represents the main research area of the Section on Neuroradiology. Our experience with one of the devices for this technique, the ACTA-Scanner, continues. We are acquiring direct clinical experience with another unit, the <u>EMI-Scanner</u>, which was installed in May of 1975 in the Clinical Center: more than 1,700 patients have been studied. A body EMI-Scanner and a second (experimental) head EMI-Scanner are being installed.</p> <p>Preliminary feasibility tests to build a totally new type of CAT device, which will use <u>protons</u> rather than x-rays, are well advanced.</p> <p>A number of <u>mathematical</u> and <u>physical</u> projects have been completed or are being carried out: review of the reconstruction algorithms; effect of beam hardening on the quality of the scan; signal vs. noise ratio; computer assisted subtraction.</p> <p>Clinical studies include CAT research on: demyelinating diseases, degenerative and atrophic processes, postradiation necrosis, spinal cord diseases, oculo-orbital lesions.</p>		

Names, Laboratory and Institute Affiliations, etc.
(Continued)

OTHER: S. P. Axelbaum	Chief Resident, Radiology	GTU
A. M. Cormack	Chairman, Physics Dept., Tufts Univ., Medford, Massachusetts	
A. M. Koehler	Director, Cyclotron Lab., Harvard Univ., Cambridge, Massachusetts	
R. N. St. Onge	Associate Professor, Physics Dept., Univ. of New Hampshire, Durham, NH	

Cooperating Units	Cyclotron Lab., Harvard Univ., Cambridge, MA;
(Continued)	Physics Dept., Univ. of New Hampshire, Durham, NH

Project Description:

Objectives: Computer Assisted Tomography (CAT) is a revolutionary diagnostic method which is having a stunning impact on the practice of neuro-radiology. This technique, which was introduced in early 1972 in England, has already been described in detail in a large number of reports in the European and American literature. At least 12 types of devices for transmission reconstructive tomography are now operational and being used in patient evaluation. In the area of emission reconstructive tomography (this actually preceded the transmission approach) one center (University of Pennsylvania) is in the forefront.

We have had direct experience with one unit for CAT, the ACTA-Scanner, for over two years. Our experience with a second unit, the British built EMI-Scanner, started in May of 1975. We have studied over 1, 00 patients with the latter unit. A second head EMI-Scanner is being installed and is being used in a few clinical studies; this unit will be employed also for experimental work in primates. A body EMI-Scanner is also being installed in the Clinical Center; in the neuroradiological field, this unit will be used for particular cranio-cerebral, oculo-orbital and spine-spinal cord problems in human patients.

Our basic experiments which will serve as a fundament to build a protons scanner (PROTO-Scanner) are far advanced.

Methods Employed: Clinical CAT scanning is now a standard diagnostic procedure.

Basic experiments being carried out as a preliminary step to build the PROTO-Scanner, involve determination in various phantoms of the absorption coefficient of the proton beam produced by a cyclotron. The phantoms include organic materials (particularly organic solutions of various concentrations).

Major Findings: In the clinical area we have:

- 1) Accumulated a significant number of cases of demyelinating degenerative

processes ("leukoencephalopathies" in the broadest sense).

2) Studied a large number of Parkinsonians and a smaller group of patients affected by Huntington's disease. An accurate evaluation of the findings in these patients will be possible when the data on the normal, age-matched, size of the ventricular system-subarachnoidal spaces is known. For this purpose a "normal control" study will soon be underway.

3) Observed interesting findings concerning postradiation necrosis of the brain. These findings may mimic brain tumors (recurrence or spread). Their recognition, therefore, is of capital importance.

4) Studied a number of patients with the so-called "atypical" teratoma syndrome. Here we have encountered findings which are quite characteristic, if not pathognomonic.

5) Introduced the concept of dynamic CAT, i.e., carried out scans before and after active or passive positional changes: supine scans followed by prone scans; scans of the eye in primary gaze followed by scans with shifted gaze.

6) Continued our work on the spinal cord. We have developed the technique of Computer Assisted Myelography (CAM). This is accomplished after the lumbar injection of metrizamide (a nonionic, hydrosoluble, radiographic contrast medium used in Europe for myelography). We have also continued our research on spinal cord cavities (syringo-hydromyelia). Particularly interesting are some preliminary results on the cord sagittal and coronal images reconstructed from axial transverse scans. A deconvolution program is used for this purpose.

In the physics area the most important finding has been the demonstration that protons can be used to detect differences in the physical properties of material at the 0.1% level. This is a significant advance when compared with the $\frac{1}{2}\%$ resolution limit of the present commercial scanners.

Significance to Bio-Medical Research and the Program of the Institute:
The diagnostic abilities in the area of neuroradiological disease are fundamentally altered by the introduction of CAT. The progress in this area is fast. Statements regarding the future significance of this methodology could be surpassed and rendered obsolete in a short time.

Proposed Course of Project: In the Section on Neuroradiology CAT will be the main area of research for years to come. We will proceed with a multi-pronged approach: theory (mathematics, physics); analytical studies; planning and building a new type of CAT device; clinical work on the brain, spinal cord and eye; experimental research on primates.

An "International Symposium on Computer Assisted Tomography in Nontumoral Diseases of the Brain, Spinal Cord and Eye," sponsored by NINCDS, will take place on the NIH campus, October 11-15, 1976.

Publications: Brooks, R.A. and Di Chiro, G.: Beam-hardening in x-ray reconstructive tomography. Phys. Med. Biol. (In Press).

Brooks, R.A. and Di Chiro, G.: Principles of reconstructive tomography in radiographic and radioisotopic imaging. Phys. Med. Biol. (In Press).

Brooks, R.A. and Di Chiro, G.: Theory of image reconstruction in computed tomography. Radiology 117: 561-572, 1975.

Brooks, R.A. and Di Chiro, G.: Statistical errors and resolution limits in x-ray reconstructive tomography. Med. Phys. (In Press).

Axelbaum, S.P., Schellinger, D., Luessenhop, A.J., Di Chiro, G., Harbert, J.C. and Twigg H.L.: Intracerebral hematoma: Diagnosis with automatic computerized transverse axial (ACTA) scanning. JAMA 235: 641-643, 1975.

Harbert, J.C., Axelbaum, S.P., Schellinger, D. and Di Chiro, G.: A preliminary comparison between ACTA-Scans and radionuclide imaging studies of the central nervous system. In DeBlanc, H.J., Jr. and Sorenson, J.A. (Eds): Noninvasive Brain Imaging: Computed Tomography and Radionuclides. New York, The Society of Nuclear Medicine, Inc., 1975, pp. 183-193

Di Chiro, G. and Schellinger, D.: Computed tomography of spinal cord after lumbar intrathecal introduction of metrizamide (Computer Assisted Myelography). Radiology (In Press).

Di Chiro, G.: Pathology of brain, spinal cord and eye recognizable by computer assisted tomography but not by traditional neuroradiology. In Proceedings of Symposium Actualitatis Tomographiae, Genoa Italy, September 11-13, 1975, Excerpta Medica, Amsterdam (In Press).

Schellinger, D., Axelbaum, S.P. and Di Chiro, G.: An analysis of the first one thousand cerebral ACTA-Scans. Fortschr. Röntgenstr. (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02201-01 SN
PERIOD COVERED <p style="text-align: center;">October 1, 1975 to June 30, 1976</p>		
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Surgical Prophylaxis for Stroke</p>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	A. K. Ommaya W. E. Lightfoote, II R. Glasser R. Uscinski A. E. Jones B. Line E. Mills D. Horwitz D. Sadowsky J. Wood R. Kaneshiro P. Fedio C. Ajmone Marsan M. K. Hammock	Actg. Chief, Surg. Neurol. Medical Officer, PHS Staff Radiologist Medical Officer, PHS Asst. Chief, Nuclear Med. Research Analyst Staff Fellow Senior Investigator Math. Statistician Medical Officer, PHS Clinical Social Worker Research Psychologist Chief, Clin. Neurosci. Senior Staff Fellow
SN NINCDS LP NINCDS DR CC SN NINCDS NM CC LA CR SN NINCDS HE NHLI OB NINCDS SN NINCDS SW CC CN NINCDS CN NINCDS SN NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Surgical Neurology Branch		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: <p style="text-align: center;">4.0</p>	PROFESSIONAL: <p style="text-align: center;">3.0</p>	OTHER: <p style="text-align: center;">1.0</p>
SUMMARY OF WORK (200 words or less - underline keywords) <p> This is a randomized, controlled clinical trial of <u>microneurosurgical anastomosis</u> between the superficial temporal artery and the middle cerebral artery for prevention of stroke in patients with <u>transient ischemic attacks</u> related to the <u>anterior cerebral circulation</u>. Patients with such symptoms are admitted for workup which includes angiography and EMI Scan and isotope studies to quantify the blood flow in the brain. Randomization between a medical arm and a surgical arm are done after diagnosing the presence and severity of transient ischemic attacks. </p>		

Project Description:

Objectives:

It is well established that the development of an adequate collateral circulation is the key factor in enabling patients to tolerate severe occlusive lesions of the cerebral circulation. Strokes occur when age and atherosclerosis render available collateral pathways inadequate. Approximately 90% of all strokes are occlusive and of these 50-80% have brief warning episodes of diffuse or focal reversible neurologic deficit, i.e. transient ischemic attacks (TIA).

A number of neurosurgeons have demonstrated the feasibility of a technique for increasing collateral CBF to the anterior circulation by anastomosing the superficial temporal artery (STA) to a cortical branch of the middle cerebral artery (MCA). This technique has now been well established as a practical surgical technique, with postoperative patency rates for the STA-MCA bypass exceeding 90% and with clinical, CBF and psychometric data showing significant improvement over preoperative levels. To date, however, no controlled study of this type of prophylactic surgery for stroke has been carried out.

This project seeks, therefore, to control the medical management and test the variable of STA-MCA surgery by randomizing it in a series of patients with anterior circulation TIA receiving uniform medical therapy. Sequentially ordered groups of patients receiving either medical therapy alone or medical plus surgical therapy will be compared for TIA and stroke incidence, pre- and post-treatment clinical status, cerebral blood flow changes and psychometric performance.

Methods Employed:

1. Clinical Testing. Detailed quantitative neurological testing will be performed before and at specified intervals after the initiation of treatment.
2. Electrophysiologic Testing. Serial electroencephalography will be carried out during outpatient and inpatient attendance. Averaged evoked potential assay of somatosensory, visual and auditory functions will be carried out before and after the treatment period at the time of the CBF estimations.
3. Isotope Scanning. Isotope scans will be carried out before and after the treatment. Post-treatment scans will be done 3 months and 12 months after initiation of therapy.
4. Computerized Tomography (EMI Scan). CT scans before and after treatment will be obtained. Ventricular capacity and visible lesions will be recorded in terms of volume using a video-planimeter developed in the Television Engineering Branch of the Clinical Center. Lesions will be identified

visually as well as by checking for numerical deviations from the norm in terms of recorded linear attenuation coefficients.

5. Angiography. Cerebral pan-angiography via a transfemoral catheter will be obtained once before and after treatment initiation. The post-treatment angiogram will be obtained prior to patient discharge, but not less than 2 weeks after treatment initiation.

6. Cerebral Blood Flow Measurement. This will be carried out before and after treatment, immediately before and after the angiography. A third estimate will be made one year after the initiation of the treatment. The technique of CBF measurement will be the noninvasive method of Austin et al. using a bolus of 10-15 mc of $^{133}\text{Xenon}$ injected intravenously, as modified in the Nuclear Medicine Department of the Clinical Center.

7. Psychometric and Social Evaluation. The patient's psychologic and social status will be evaluated before, one month after, and one year after the treatment by a Clinical Psychologist and a Social Worker.

8. Each patient will be assigned to one of two categories, the first receiving continuing medical therapy alone, the second receiving a TCA-MCA anastomosis plus medical and appropriate supportive therapy.

9. Surgical Technique. This will be as described by Reichman and others.

Major Findings:

To date 27 patients have been referred to the study, of which 3 have been admitted for further investigation. One of these cases is ready for randomization. It will take some time to develop an adequate number of cases for this study.

Significance to Biomedical Research and the Program of the Institute:

The data on cerebral blood flow and computerized tomography of these patients will be of value in understanding strokes and cerebrovascular diseases. The value of a surgically attractive technique will be established.

Proposed Course:

It is proposed to attract a Senior Investigator for this project via the IPA mechanism. Experience to date has shown that only a full-time effort will enable the number of cases needed for the project to become adequate.

Publications:

None

ANNUAL REPORT

July 1, 1975 through June 30, 1976

Laboratory of Central Nervous System Studies

National Institute of Neurological and Communicative Disorders and Stroke

The Laboratory of Central Nervous System Studies under the direction of D. Carleton Gajdusek, M.D. comprises two major projects: (1) Neurobiology of Population Isolates--the Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures; and (2) Chronic Central Nervous System Disease Studies--Slow, Latent and Temperate Virus Infections. Both projects are an outgrowth of the Study of Child Growth and Disease Patterns in Primitive Cultures. It was this parent organization that gave rise to the discovery of kuru, a heredofamilial subacute progressive degenerative disease of the central nervous system of the Fore people in the Eastern Highlands of Papua New Guinea. It was this study that led to the demonstration that kuru is caused by a serially transmissible virus which possesses unconventional biological and biochemical properties. It was the successful transmission of kuru and the isolation of the virus which provided the necessary techniques for the subsequent discovery of a viral etiology for some forms of presenile and senile dementias of man, particularly the Creutzfeldt-Jakob type and more recently certain forms of familial Alzheimer's disease and progressive supranuclear palsy.

These discoveries made possible the recognition that Creutzfeldt-Jakob disease may be transmitted by transplantation surgery, e.g. as in the case of corneal grafts that occurred accidentally in the United States, warranting just concern for possible contamination in all tissue transplantations from donors dying with presenile and senile dementias. Moreover, they have led to the demonstration that the virus of Creutzfeldt-Jakob disease remains viable and can be recovered from human tissue that has been preserved in 10% formalin for more than 8 months. It has also been possible to identify the virus of transmissible virus dementia (CJD-type) as the cause of death in a neurosurgeon and to raise the possibility of occupational hazard. These findings enabled us to apply our research data to the very real need for caution on the part of neurosurgeons and neuropathologists in the area of occupational hazards in handling tissues from CJD patients. In the past year, with our demonstration of the transmissibility of scrapie disease from American sheep and English goats to several species of non-human primates, manifested by a disease in the experimental monkey which is indistinguishable from the transmissible virus dementia originating from man, we are confronted with the urgent question of the possible relationship between scrapie of sheep and the spongiform encephalopathies of man.

The elucidation of the etiology and epidemiology of a rare, exotic disease restricted to a small population isolate--kuru in New Guinea--has now brought us to worldwide considerations that have importance for all of medicine and microbiology. For neurology, specifically, we have considerable new insights into the whole range of presenile dementias, and, in particular, to the larger problems of Alzheimer's disease, familial and senile dementias,

and the processes of CNS aging. The implications of vertical transmission of slow virus infections, of conjugal transmission of these diseases, and of host genetic control of disease expression for all genetic diseases, and the relationship of these slow virus infection processes to those which may lead to neoplastic transformation are obvious.

The major problems among the degenerative diseases of multiple sclerosis, amyotrophic lateral sclerosis, and Parkinsonism remain unsolved although there are tantalizing laboratory and epidemiological data pointing to the possible role of virus-like agents in these diseases. Perhaps the masked and defective slow infections with conventional viruses such as are seen in PML and SSPE may provide the best leads for studying these diseases.

The foci of high incidence of amyotrophic lateral sclerosis with associated high incidence of parkinsonism-dementia complex among the Chamorro people on Guam and the Japanese of the Kii Peninsula remain continuing challenges. Our discovery and re-evaluation, during the 1974, 1975 and 1976 field trips to the South Pacific, of the very small but very intense foci of such motor neuron diseases with associated high incidence of Parkinsonism, parkinsonism-dementia, and other peculiar bradykinetic and myoclonic dementia syndromes among the Auyu and Japai people in a remote population of West New Guinea, suggests strongly that some common etiological factor may underlie the occurrence of all these very different syndromes, as they occur strangely in this one small population and are not found in the much larger surrounding populations. This ALS-PD focus was extended as a result of the 1976 patrol to Ganda. Several additional villages in West Irian have now been added to the growing list of foci of ALS-PD with deaths occurring since 1965. A program of demographic and epidemiologic field work to intensively study these foci has already been designed and will be conducted within the upcoming fiscal year. This will require 4-6 months of intensive field study.

The models of lysogenicity and of subviral genetically active macromolecular structures from the study of bacterial viruses and bacterial genetics supply ample imaginative framework for an expression of our ideas of possible mechanisms of infectious pathogenesis in man. The unconventional viruses of the spongiform encephalopathies tax even our imagination in relation to molecular biology gained from these studies in bacteria.

For a now-disappearing disease, kuru, in a small primitive population to have brought us this far is ample reason for pursuing intensively the challenges offered by the still inexplicable high incidence and peculiar profusion of different neurological syndromes, pathologically distinct yet apparently somehow related to each other, which have been discovered in the several small population enclaves we have investigated. Thus, the high incidence of ALS, ALS-PD on Guam and among a small population of people in West New Guinea, coupled with the high incidence of ALS on the Kii peninsula of Japan may indeed offer the best opportunity of solving the problem of this sclerosing disease which in the United States has an incidence as high as that of multiple sclerosis.

The delineation of infection as the etiology of hereditary and presenile and senile dementias of man was made possible only through the concomitant studies of the neurobiology of population isolates. In this area we have been engrossed in the investigation of deaf-mutism, mental subnormality and other congenital central nervous system defects associated with endemic goiter in the Central Highlands of Western New Guinea, as well as patterns of delayed puberty, slow growth rates, and of early aging in isolated Melanesian groups. Ethnic drug abuse (particularly of kava), strange patterns of psychosexual development, pseudohermaphroditism, and culturally-determined responses to pain, and roots of aesthetic expression, have all been under study. Foci in primitive population isolates of familial periodic paralysis, progressive muscular dystrophy (both the pseudohypertrophic type of Duchenne and the non-pseudohypertrophic distal type), amyotrophic lateral sclerosis and Parkinsonism, are also being investigated. Genetic studies on human evolution led to the discovery of new genetic factors among haptoglobin, hemoglobin, and red cell enzyme pleomorphisms and the definition of their biochemical structure.

The further significance of scientific investigations of small population enclaves of remote populations has been even more dramatically apparent during the 1975-1976 field trip with our re-evaluation of what may turn out to be one of the largest "epidemics of epilepsy" ever recorded. This is occurring in the Wissel Lakes area of West New Guinea and is the result of cysticercosis, with the larvae of Taenia solium, the pig-tape worm, which has been newly introduced into New Guinea. Our recent studies have led us to conclude that the natural history of cysticercosis epilepsy is not a result of death of the worm, scarring and calcification of lesions, as much of the literature suggests, but is an early sign in inflammation from new invasion of the brain by the Taenia larvae. First, convulsions often occur even before the first subcutaneous nodules appear, and as the nodules increase in number, additional seizures occur. The high incidence of severe third degree burns, which may even result in death, is a direct result of cysticercosis-induced unexpected seizures which occur during the sleeping hours, throwing the patient into the house fire. The unclothed people, living at 2000 meter elevation, need to sleep close to the home fires on cold nights. We are able to date the first introduction of Taenia solium into the area and to plot the spread of Taeniasis in pigs and man, and of cysticercosis and associated epilepsy in man to other previously Taenia-free areas. With Dr. Budi Subianto, the local Indonesian Medical Officer, we have planned a neuroepidemiologic study aimed at elucidating the natural history of the epilepsy and acute psychoses and other neurological complications which have occurred concomitantly with the emergence of subcutaneous cysticercosis nodules.

Dr. Gajdusek has been invited by Soviet investigators as the consultant to their study of the huge focus of a chronic degenerative basal ganglia extrapyramidal disorder called Vilyuisk encephalitis, which is restricted to the Yakut people in eastern Siberia. For his visit, many investigators, including the Minister of Health of the Yakut Republic, were brought to Moscow, along with four patients suffering from the advanced stages of the disease. There were three days of presentations of the field epidemiological, clinical and laboratory data which had been accumulated, and several hours of discussions on the direction the future studies should take. The disease is a

syndrome which has not been previously encountered elsewhere. There is a high possibility that it is a slow virus infection. Our Soviet colleagues have indicated that they will invite us to participate in future meetings devoted to this disease.

The development and maturation of the two major projects of this laboratory resulted from cross-fertilization of each since their origin, and both have grown from the basic studies on child growth and development and disease patterns in primitive cultures. And, although the two projects, each composed of many subsections, differ markedly in the inquiries they phrase and the techniques of investigation they employ, much of the field data collected from one project is also requisite for the studies in other projects. Both are served by the same investigators who function as a team. These scientists derive their creative stimulus, dedication and enthusiasm, to a great extent from the atypical and exotic biological, social, and cultural materials presented and the diverse, frequently unconventional approaches phrased by the two projects.

Principal Investigators: D. Carleton Gajdusek, M.D.
Clarence J. Gibbs, Jr., Ph.D.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01282-12 CNSS	
PERIOD COVERED July 1, 1975 through June 30, 1976					
TITLE OF PROJECT (80 characters or less) Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PRINCIPAL INVESTIGATORS: D. Carleton Gajdusek, M.D., Chief, LCNSS; Clarence J. Gibbs, Jr., Ph.D., Assistant Chief, LCNSS; and Paul W. Brown, M.D. Michael Alpers, M.D.; David M. Asher, M.D.; Richard Benfante, M.A.; Peter Fetchko, M.A.; Josede Figirliyang, M.A.; Ralph Garruto, Ph.D.; Chev Kidson, M.D.; Robert L. Kirk, Ph.D.; Ivan Mbagintao; Judith Meyer; Steven Ono; Donald Rubinstein; Roger Traub, M.D.; Stephen Wiesenfeld, M.D.; Richard Yanagihara, M.D.; Vincent Zigas, M.D. Jacques Bert, M.D.; Francoise Cathala, M.D., Louis Court, M.D.; Arwin R. Diwan, Ph.D., Lydia Fadeeva, M.D., Robert MacLennan, M.D.					
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LAB/BRANCH (continued) Laboratory of Central Nervous System Studies, Intramural Research Program					
SECTION					
INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20014					
TOTAL MANYEARS: 12		PROFESSIONAL: 8		OTHER: 4	
SUMMARY OF WORK (200 words or less - underline keywords) Studies of human biology of vanishing <u>primitive societies</u> focus on <u>neurological development</u> and <u>learning</u> patterns in diverse cultural experiments in the <u>human condition</u> found in such isolated groups. Laboratory studies on <u>population genetics</u> , molecular biology, immunology, virology, endocrinology and biochemistry are aimed at problems more appropriately studied in small isolated primitive bands than in civilized societies. Data and specimens collected over years on expeditions to <u>Micronesia</u> , <u>Polynesia</u> , Solomon Islands, New Hebrides, <u>New Guinea</u> , Indonesia, <u>S. America</u> and Asia are used. Studies on nutrition, reproduction, fertility, neuroendocrine influences on age of <u>sexual maturation</u> and <u>aging</u> , genetic polymorphisms, genetic distance, unusual and odd employment of the higher cerebral CNS function of <u>language learning</u> , cognitive styles, computation (calculation without words or numbers), and culturally modified <u>sexual behavior</u> elucidate alternative forms of neurologic functioning for man which we would be unable to investigate once the natural cultural experiments in primitive human isolates were amalgamated into the cosmopolitan community of man. Foci of high prevalence <u>kuru</u> , <u>ALS</u> , <u>parkinsonism-dementia</u> , myoclonic <u>epilepsy</u> , kuru-like syndromes, <u>hysterical disorders</u> , <u>schizophrenia</u> , periodic paralysis, <u>muscular dystrophies</u> , congenital defects, <u>goiter</u> , <u>cretinism</u> , deaf-mutism, cancers, tropical ulcers, diabetes, <u>pseudohemaphroditism</u> , <u>albinism</u> , familial rheumatoid arthritis, amyloidosis, asthma, chronic lung disease, <u>epilepsy</u> , <u>cysticercosis</u> , taeniasis, filariasis, leprosy and <u>acute infections</u> are investigated.					

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- Sub-Project I: Study of the developmental patterning of the human nervous system (cybernetics of human development).
- Sub-Project II: Human evolutionary studies in isolated primitive groups.
- Sub-Project III: Studies of isolated Micronesian populations.
- Sub-Project IV: Studies of isolated New Guinea populations.
- Sub-Project V: Studies of Australian Aborigines.
- Sub-Project VI: Studies of isolated New Hebrides and Solomon Islands populations.
- Sub-Project VII: Studies of Central and South America Indians.
- Sub-Project VIII: Developmental, genetic and disease patterns in primitive populations of Asia, Africa, Indonesia, Melanesia, Micronesia, Polynesia and the Arctic.
- Sub-Project IX: Experimental developmental neuropsychiatrics in infantile programming; an empirical approach to the language of information input into the nervous system.
- Sub-Project X: Ciphers and notation for the coding of sensory data for neurological information processing.
- Sub-Project XI: Racial distribution and neuroanatomic variations in the structure of the human brain.
- Sub-Project XII: Studies of high incidence of neurological diseases in specific racial and ethnic groups and in primitive or geographic population isolates.
- Project Description: Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures (described fully on pages 11r through 18r)
- Publications: Listed on pages 40r through 44r.

Study of Child Growth and Development,
Behavior and Learning and Disease Patterns in Primitive Cultures

Our goal has been to increase our understanding of human population biology in the areas of neurobiology, immunology, genetics and the study of behavioral patterns and diverse styles and modes of learning associated with natural experiments in remote, isolated, technologically simple human population groups throughout the world. Our overall research program in this area has been directed towards problems of neurological development and learning patterns in children from diverse cultural settings which are found in these isolated primitive populations. Laboratory studies on human biology, genetics and associated molecular biology, on immunology, virology and biochemistry, and on biological adaptation to diverse and extreme environmental conditions have all been directed at solving problems which have been carefully chosen from small isolated bands still living in the primitive situation in which these problems may be more appropriately studied than in the larger civilized societies. Nutritional studies, studies on reproduction and fertility, use of drugs on neuroendocrine influences on age of sexual maturation and aging, and studies of the selective advantage and the establishment of population equilibrium with genetic polymorphisms are under way. The unusual and odd alternative methods of employing the central nervous system in its higher cerebral function of patterns of language, cognitive style, computation (number sense and calculation without a number system), and psychosexual culturally-modified behavior, and methods of visual, auditory and tactile learning are providing data on alternative forms of possible neurological functioning for man. We should remain unaware of such alternative forms of neurological functioning, and be unable to produce or investigate them in the clinic or laboratory, once the natural cultural experiments in primitive human population isolates were finally amalgamated into the modern civilized cultural veneer which is now imposed on almost all members of the community of man.

During the expedition to West New Guinea this year, our knowledge of the focus of an extremely high incidence of ALS with associated parkinsonism and dementia syndromes was greatly extended. New cases have continued to appear with undiminished frequency in a group of villages of the Jaqai and Auyu cultures with a total population of about 5000. The incidence of ALS in these affected villages is over one thousand-fold that usually seen in Europe, Japan, Australia, and America and is thus considerably higher than the high incidence found among the Chamorro people on Guam and the Japanese in the Kii peninsula. Further examples of familial incidence were found. The perimeter of the area containing the affected villages was more accurately determined and a wider area of surrounding populations was surveyed for all neurological diseases. The demographic study of the affected and unaffected surrounding populations was expanded. The interesting conclusions already possible are: (1) there is no chance that genetic mixing has occurred between these populations and other populations of known high incidence; (2) the associated high incidence of parkinsonism and dementia syndromes with high incidence of motor neuron disease, and the absence of these latter syndromes in the

villages in which motor neuron disease is not found, recapitulates this interesting association found among the Chamorro on Guam and the Japanese on the Kii Peninsula.

This year our section has for the first time been extensively involved in the reassessment of the epidemiological and genetic investigation of the high incidence ALS-PD on Guam among the Chamorro peoples. In the past, our attention to this focus has been restricted to laboratory investigations on specimens provided from the field team there. We have completed and have underway red cell typing and serological testing of serum from patients for their immune status to a large number of viruses and other microbial agents, and this is being controlled by an extension of our long-standing sero-epidemiological investigations on the Chamorro and other Micronesian populations. Blood genetic studies, including blood groups, 24-red cell enzyme systems and serum protein systems, as well as HLA typing and mixed leukocyte agglutination on patients and on control subjects are under way. We already know that there is no high level association of either ALS or PD syndromes with HLA type, but at a low level, interesting associations are becoming apparent. The establishment of our tissue culture laboratory for a period of several months on Guam, during which brain cell and visceral cell and skin biopsy tissue cultures were established on many patients and sent back to the NIH, will be discussed in the report of Chronic CNS Disease Studies (pages 25r through 46r).

Return epidemiological investigations in the kuru region perhaps offer our best chance of discovering the mode of transmission and biological maintenance of one of the unconventional viruses. The mode of transmission, dissemination and maintenance of the agent of the transmissible virus dementia (Creutzfeldt-Jakob disease) has eluded us. Strangely, that of kuru seems perfectly and totally elucidated. Kuru has continued to disappear with fewer deaths each year: 63 in 1973; 54 in 1974; 35 in 1975; and currently five patients have died in 1976, with 17 living patients now under study who may die before the end of this year. Every year the youngest patient is a year or more older; no person born since cannibalism ceased in his village has died of kuru. There is strong epidemiological evidence against transplacental or neonatal transmission, since of several hundred children born during active kuru disease to patients studied before 1963, none have developed the disease. Our experience with kuru in South Fore children in the early years of investigation would have led us to expect several dozen such kuru deaths already in childhood in this series. Thus, transmission to her fetus or infant during pregnancy and parturition in a kuru patient is not the source of kuru in childhood. With the current vast decrease in the incidence and prevalence of kuru, it is now possible to plot "bursts" of kuru patients who are related by co-residence two or more decades earlier in a household in which a kuru death and the cannibalistic consumption of the kuru victim by kinsmen occurred. Such "bursts" indicate an incubation period of 20 years or more. This year, within such "burst" episodes, two instances of simultaneous kuru in siblings have been discovered. Thus far, no case of kuru has been seen in an individual born and nurtured outside the kuru region and no contact case has developed in an immigrant into the region since cannibalism and warfare ceased. Dr. Michael Alpers, working with our section, is currently continuing the field epidemiological study of kuru in New Guinea.

During previous and the current field studies in Papua New Guinea and in West New Guinea (Irian Jaya, Indonesia), and smaller Melanesian Islands, focal degenerative neurological diseases have been found, and further studied in numerous isolated populations. Foci under study include: (1) a strangely severe form of familial periodic paralysis with associated hypokalemic complete heart block on the island of Tongariki in the New Hebrides; (2) a large family with pseudohypertrophic muscular dystrophy, deaf-mutism, and feeble-mindedness among the Nakanai people of West New Britain; (3) an extended family with a distal form of non-pseudohypertrophic muscular dystrophy in the Sentani Lake region of West New Guinea; (4) a family with virtual blindness produced by extreme congenital hypersensitivity to light in the absence of albinism among the Tjitak people of West New Guinea; and (5) several other varieties of familial mental deficiencies in different Melanesian groups. The family with pseudohypertrophic muscular dystrophy, deaf-mutism and feeble-mindedness demonstrates complete dissociation of each syndrome of this triad from the other two in one or more individuals; other individuals show two or all three of these abnormalities. The family with distal non-pseudohypertrophic muscular dystrophy has had one individual with an unusual malignant course lasting less than one year to death.

The discovery last year of a case of Creutzfeldt-Jakob disease (proved by the examination of a brain biopsy) in a New Guinea Highlander living far outside the kuru region has added strength to the speculation that kuru may have originated by the rapid man-to-man passage through cannibalistic ritual of CJD virus from a sporadic case of the disease in a Highland New Guinean. Laboratory evidence of the rapid change in the virulence properties of the virus by the mode of passage support this hypothesis. It is of interest that the presence of CJD and of kuru in Highland New Guineans cannot be linked to sheep or goats carrying the scrapie virus, for both of the human diseases have occurred before these animal species were introduced to the affected peoples.

This year we returned to survey the "epidemic of epilepsy" in the Wissel Lakes area of West New Guinea, which Drs. Subianto and Tumada and Gunawan of the Irian Jaya Department of Health are studying. The epilepsy had resulted from the introduction in 1971 of the pig-tapeworm, Taenia solium, with the importation of new pigs for breeding stock from western Indonesia. The New Guinea Highlands were known to be free of Taenia solium in pigs and man prior to this, and current surveys indicate that they are still free, with the exception of the focus spreading from the Wissel Lakes region.

The introduction of Taenia solium was first recognized by the natives themselves on butchering their pigs, when they found for the first time the larval cysts producing "measly pork". Within a year, Dr. Tumada discovered intestinal taeniasis in over ten percent of the school children and subsequently intestinal taeniasis has increased in frequency to over twenty percent in some regions, and has gradually spread to involve the whole Ekari population (some 60,000), and some of the adjoining populations.

About one year after intestinal taeniasis in man (eggs and proglottids in stools) was discovered, subcutaneous nodules began to be noted by the natives themselves as a phenomenon new to them. On surgical excision of the

troublesome nodules, both the natives and doctors were immediately aware that they had the same disease as their pigs. This has been substantiated by parasitological study. Such cysticercosis is the result of consuming Taenia solium eggs from human fecal contamination and not from eating measly pork. During the last three years an intense epidemic of severe burns has occurred in adolescents and adults, unlike any seen in the previous three decades of medical experience in the Highlands. The almost naked people, living at 2000 meters elevation, find it necessary to sleep huddled close to the house fires to keep warm at night. Severe, sometimes fatal burns have occurred as the result of grand mal convulsions occurring at night in people with no previous history of epilepsy. Many of the several hundred new epileptics whom the doctors have seen have had their first seizure and resulting severe burns--many requiring amputation--before any subcutaneous nodules diagnostic of cysticercosis had appeared. Subsequently, during convalescence from their burns, further seizures and the new appearance of subcutaneous cysticercosis nodules have been noted in many patients. These observations suggest that the cysticercosis induced epilepsy is occurring during the early stages of larval invasion, rather than late in the disease, with death of the larval and resulting inflammation, sclerosis and calcification of the cysts, as reported in the literature. This is further substantiated by the unique opportunity this epidemic has afforded to study the natural history of cysticercosis-induced epilepsy. A well-planned epidemiological and clinical study of the neurological disease is under way. It is already evident that after one or a few further seizures, most patients are having no further seizures or residual neurological signs or symptoms. A few patients, however, have progressed from their first seizure to severe generalized debilitating neurological disease, coma and death. The brain of one such autopsied child, loaded with large cysts, is under neuropathological study. Some patients are also displaying acute psychosis, with dangerous violent behavior concomitant with the emergence of subcutaneous cysticercosis nodules, and this is obviously another form of cysticercosis with CNS involvement. Some such psychotic patients also have convulsions; others do not.

The evaluation of a focus of male pseudohermaphroditism in the New Guinea Highlands has continued with an eighteen-year perspective on the problem, which we discovered and first reported two decades ago. The syndrome results in infants and children reared as girls changing to obvious males in habitus and partially so in genital appearance with puberty. This year the field expedition in a very remote area of the Highlands of New Guinea made possible the complete delineation of the focus of this disorder among the Anga people, and a summary of the clinical and sociological aspects of the problem is currently in preparation, with over a decade of longitudinal studies on a number of patients. The focus consists of more than a dozen patients, eight of whom have been studied in detail, and genetic and clinical laboratory investigations are under way. The focus appears to be similar to the one discovered recently in the Dominican Republic by Dr. R.E. Peterson of Cornell University, involving steroid 5- α -reductase deficiency. Biochemical studies to determine this issue are now in progress.

Studies on malarial antibody are now completed from the extensive serological testing of sera collected on our U.S. Research Vessel Alpha Helix

expedition to the New Hebrides and Solomon Islands in 1972. Results indicate that fluorescent antibody test for malaria has very limited usefulness in defining the malarial species prevalent in an endemic area and has emphasized the necessity for more specific reagents (e.g. to each malarial species) for this test to be of any practical value in malarial seroepidemiology.

Studies on SV-40 and BK virus are continuing, with the examination of pre- and post-infection sera from humans inoculated with SV-40 virus in the late 1950's, and show that documented SV-40 infection in man does not induce heterologous antibody to the related BK papovavirus.

We have documented and will soon publish the very early acquisition of CMV and EBV virus in the remote isolated human populations of the South Pacific. Almost 100% of all subjects under four years of age were seropositive for both viruses; of 16 subjects under two years of age all were seropositive. This is attributed to the close interpersonal contact between a single infant and the many individuals who handle him, a cultural practice which exists in the open expanded family setting, as contrasted with the Western nuclear family, where only a few close family members handle and feed the infant and, thus, reduce the possibility of the child's handler being the spreader of herpes virus.

We have completed testing several hundred sera from Highland Quechua Indians of Peru for immunological response to various respiratory viruses. In this hypoxic environment of altitude (14,000 feet), the major cause of death is respiratory complications in both young and old; such disease stress is more emphatic in an oxygen-deficient environment and we are studying ways in which this population biologically adjusts to such circumstances with very little Western medical practices existing, and with no cultural and technological modifications of hypoxic stress possible.

A study of Group A arboviruses in the South Pacific has recently been completed and published in collaboration with Dr. L. Rosen and Dr. R. Tesh of NIAID, in which we and the Hawaii laboratory have elucidated the extent and range of these viruses from Southeast Asia to Micronesia, New Hebrides, the Solomon Islands and New Guinea. Under investigation currently are the Group B arboviruses, the results of which will be published this year.

Studies of Clostridium tetani are in progress to determine if natural immunity exists in isolated human populations throughout the world. In view of the recent indications that a natural immunity to the toxin may exist, a worldwide serological survey of isolated populations which are without prior significant contact with people outside their areas or with immunization programs is currently being conducted.

During the course of investigations of kuru and other subacute and degenerative neurological disorders, long-term studies of the human biology of the affected populations and non-affected control populations were undertaken toward elucidating the causes and etiological factors contributing to the expression of these diseases. The major groups under study are Melanesian natives living as population isolates in the Eastern Highlands of Papua New

Guinea. These investigations have included surveys of such populations to determine the spectrum of disease incidence, and to assess the present and past impact of various diseases on these populations. Field evaluations are supplemented by serological tests, including complement fixation, neutralization, hemagglutination inhibition and fluorescent antibody determinations to detect the presence of specific antibody to a wide range of microbial pathogens. Such seroepidemiological studies of primitive isolated populations in conjunction with other epidemiological, ecological, genetic, immunological and biochemical data provide unique opportunities for investigating the pathogenesis and etiology of many diseases in a situation more amenable to analysis than is the complex situation that exists in advanced cosmopolitan communities. A series of papers are underway, some are already published, which report the results of the seroepidemiological survey of the Eastern Highlands of Papua New Guinea, West New Guinea and Micronesia. These include studies on the incidence and age distribution of infections with bacteria and viruses, rickettsiae, parasitic helminths and protozoa, mycoplasma, treponemata, leptospirae, Bedsoniae, and fungi.

Potent tools of mathematical population genetics have been used with our massive genetic pleomorphism data on primitive groups for the analyses of the multilocus genetic effects in determining expression of neurological diseases such as kuru and a similar approach is under way for the transmissible virus dementias. Genetic distance techniques, including genetic tree construction and principal components analyses are also used. From populations in the Eastern Highlands of New Guinea, red cells, serum and leucocyte types have been analyzed for major genetic polymorphic systems. These include ABO, MNS, Rh, Kell, Diego, Duffy, Wright, Lewis and P blood group systems; serum protein polymorphic factors: transferrins, haptoglobins, albumin, group-specific protein (Gc) and Gm and Inv allotypes of immunoglobulin G, and many red cell enzymes. Such genetic studies are used to test for degrees of population isolation, drift, migration, and unusual gene frequencies as might occur. The possibility of detecting associations of genetic factors with disease underlies much of the motivation of this quest. For kuru and ALS-PD, as in multiple sclerosis and ankylosing spondylitis, there is already some success in this direction.

Growth and development studies in primitive New Guinea cultures have yielded evidence for delayed puberty, slow growth rates, and early aging in certain peoples, particularly for the short-statured populations in the Highlands of New Guinea. Preliminary evidence suggests high levels of pituitary growth hormone from the pituitary glands obtained at autopsy, and in serum specimens. The growth rate seems to be proportional to, and the age of puberty inversely proportional to the mean adult stature. Extreme early aging of a small subset of individuals in several Highland cultures, associated with small stature and later puberty, is under investigation.

Our efforts to document the development and neurological patterning in the disappearing primitive cultures has resulted in one of the largest archives of such documentation in the world. The collection and preservation of cinema data from New Guinean, Oceanian, African, Australian aborigine and American Indian groups as they live as hunter-gatherers or primitive hoe and

digging stick agriculturists, provided for many years the only such documentation of the life and behavior of man as he lived and evolved for most of his evolutionary history of about three million years.

This section has now been instrumental in establishing and financing the National Anthropological Film Center at the Smithsonian Institution. The Director of this new national facility was transferred to the Smithsonian Institution from this NINCDS project, where he was trained and had worked for over a decade. The large section of research films which form the nucleus of that new Smithsonian Archive of the National Anthropological Film Center, were films transferred from the NINCDS Archive where all of the research films for this new facility had been prepared. The methods and procedures for documenting and of preparing and preserving research films developed in this section are now generally in use in that Archive, and elsewhere.

Currently, studies using cinema recordings of diverse tremors and movement disorders in culturally, ethnically and geographically isolated populations are in progress. Similarly, the study of chronological progression of Creutzfeldt-Jakob disease, kuru and ALS-PD, and muscular dystrophy is being attempted by comparisons of cinema records during different stages of the disease in a given patient.

Cinema analysis of hysterical equivalents or dissociation syndromes and other "infectious", often epidemic movement disorders akin to amok, koro, lulu, latah, sangtaingua and "wild man" behavior in Melanesian, Micronesian and Malaysian populations is underway as part of a major study of culturally patterned and determined modes of psychological breakdown. Many of these syndromes closely resemble epilepsy, tetanus, kuru and other neurological movement disorders. The mechanisms of copying and mimicking and reenacting patterned behavior involved in these culturally specific syndromes, suggest parallels in such widely diverse phenomena as those of sighting unidentified flying objects; faddish phenomena, such as panty raids, summer riots, sudden changes of tonsorial and dress style; fashions of hijacking, sit-ins, drug abuse, and epidemic suicides, kidnappings or assassinations; tarantula, shakers and other dance manias; confessions of witchcraft; consumer purchasing; and many other follow-the-leader social phenomena. Faddish and fashionable pursuit of goals, techniques or ideas, is only too evident in our own fields of research as well. That they may have an understandable neurophysiological substratum, as Paul MacLean's studies indicate, in echolalia, echopraxia and suggestibility in hysteria patients is entertained.

Our many and diverse investigations have evolved about the theme of elucidating the medical problems in the cultures of primitive man, or man living in small isolated communities, by carefully selecting problems of immunological, virological, biochemical, genetic, reproductive biological, endocrinological, nutritional and psychiatric interest which are pertinent to long-term studies of growth, behavior, emotional responses to stress and human biology and disease pattern in these groups. This opportunistic use and study of such "natural experiments" has already resulted in major contributions to microbiology, molecular biology, genetics, biochemistry, endocrinology and other basic sciences and also to many clinical disciplines of importance far

beyond the application of the findings to the problems encountered in the primitive or isolated communities studied. The value of these findings to the people under investigation, suffering inordinately from the disorders that have focused our attention, is obvious.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 00969-12 CNSS
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PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Chronic CNS Disease Studies: Slow, Latent and Temperate Virus Infections

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH

(continued)

Laboratory of Central Nervous System Studies, Intramural Research Program

SECTION

INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders
and Stroke, National Institutes of Health, Bethesda, Maryland 20014

TOTAL MANYEARS:

24

PROFESSIONAL:

14

OTHER:

10

SUMMARY OF WORK (200 words or less - underline keywords) Studies elucidate cause and pathogenesis of chronic degenerative CNS disorders with emphasis on MS, ALS, parkinsonism-dementia, Parkinson's, Pick's and Alzheimer's diseases, Huntington's chorea, supranuclear palsy, other presenile dementias, chronic encephalitis with focal epilepsy, muscular dystrophies, chronic schizophrenia, SSPE, PML, dialysis encephalopathy, and intracranial neoplasms. Even familial, apparently hereditary diseases may be slow virus infections. Subacute spongiform virus encephalopathies (kuru and Creutzfeldt-Jakob (CJD) diseases of man; scrapie and mink encephalopathy) are caused by unconventional viruses with unique properties posing important theoretical problems to microbiology and molecular biology; a major goal is elucidation of their structure and mechanisms of replication. Transmissible virus dementias are increasingly recognized worldwide causes of death: high incidence foci, transmission by corneal transplant, and occupational hazards from exposure to human brain in surgery or pathology are found. Scrapie transmitted to primates causes disease indistinguishable from experimental CJD. Zonal UC, electrophoretic, chromatographic purification, UV and ionizing radiation inactivation, EM freeze-fracture membrane studies on scrapie are under way. Finding many latent oncogenic viruses, including reverse transcriptase producing oncornaviruses in healthy primate brains and slowly developing noninflammatory pathogenic effects from persistent, masked and defective infection suggest relationships to the cancer problem.

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- Sub-Project I: Attempts to isolate, identify and characterize transmissible agents from humans and animals with subacute degenerative diseases of the central nervous system: transmissible heredofamilial diseases, pre-senile and senile dementias of the sporadic and familial types and primary sclerosing and demyelinating diseases.
- Sub-Project II: Characterization and pathogenesis of kuru virus.
- Sub-Project III: Characterization and pathogenesis of Creutzfeldt-Jakob disease (transmissible dementia) virus.
- Sub-Project IV: Scrapie: studies on the purification, physical and biological characterization and nature of the virus.
- Sub-Project V: In vivo and in vitro host range of the subacute spongiform virus encephalopathies.
- Sub-Project VI: Isolation and characterization of adenovirus from the urine of chimpanzees.
- Sub-Project VII: Fluorescent antibody studies on the intracellular localization and identification of viral antigens in vivo and in vitro in tissues from patients with subacute diseases of the CNS.
- Sub-Project VIII: Tissue and cell culture in vitro studies of virus induced slow infections of man and animals.
- Sub-Project IX: The syncytium forming viruses (simian and human foamy viruses).
- Sub-Project X: Studies on transformed human brain tissue in vitro and characterization of associated virus.
- Sub-Project XI: Electron microscopic membrane studies of subacute spongiform virus encephalopathies.
- Sub-Project XII: Characterization and identification of new herpes viruses from explant cultures of tissues from sub-human primates.
- Sub-Project XIII: Studies on persistent asymptomatic cytomegalovirus infections of healthy rhesus monkeys.
- Sub-Project XIV: Focal movement disorders in rhesus monkeys following experimental infection with a strain of tick-borne encephalitis virus.

- Sub-Project XV: Development of serological and immunological test system for use in the study of slow infections of the CNS.
- Sub-Project XVI: Immune responsiveness of multiple sclerosis patients to established viral antigens by detection of specific antibodies in serum and cerebrospinal fluids collected serially during remission and exacerbation.
- Sub-Project XVII: Animal management and intercurrent diseases in sub-human primates on long-term studies of slow infections.
- Sub-Project XVIII: Studies to determine the possible presence of cryptic viral genomes in human brain tissues.
- Sub-Project XIX: Sequential development of kuru induced neuropathological lesions in spider monkeys.
- Sub-Project XX: Studies on the isolation, characterization, identification and pathogenicity of type C viruses from human and animal tissues.
- Sub-Project XXI: Biochemical studies of the etiology of amyotrophic lateral sclerosis and Parkinsonism dementia.
- Sub-Project XXII: In vitro transformation of CNS cells persistently infected with subacute spongiform virus encephalopathies.
- Sub-Project XXIII: Study of mitochondrial mutants from scrapie infected mouse brain cells.

Previous Serial Number: NDS (I)-65CNSS 969

Project Description: Chronic Central Nervous System Disease Studies (described fully on pages 26r through 39r).

Publications: Listed on pages 40r through 44r.

The projects (I through XXIII) listed herein, as itemized in the Project Reports of previous years, have continued throughout this year and have been expanded, as are reflected in the extensive list of publications and the summary in pages 26r - 44r. Contractural phases of this work are being conducted at:

Gulf South Research Institute, New Iberia, Louisiana	\$ 445,000
Public Health Research Institute of New York, Otisville	74,000
Center for Primate Biology, University of California, Davis, California	100,000
Department of Molecular Biology, University of Connecticut	101,000

CHRONIC CENTRAL NERVOUS SYSTEM DISEASE STUDIES:

Study of Slow, Latent and Temperate Virus Infections

Viral diseases have generally been considered acute and self-limiting, leading quickly to either death or recovery. Chronic diseases not otherwise classifiable have long been labeled as degenerative. We have now proven that certain types of heredofamilial, presenile and senile dementias of the kuru, Creutzfeldt-Jakob disease and familial Alzheimer's disease types are slowly evolving infections of the central nervous system induced by viruses. These viruses have unusual biological and biochemical properties and we thus refer to them as the "unconventional" viruses belonging to the group subacute spongiform encephalopathies. Other central nervous system diseases associated with "slow infectious processes", such as progressive multifocal leucoencephalopathy and subacute sclerosing panencephalitis, are caused by the more conventional type viruses such as papovaviruses (JC and SV-40), measles, and rubella. Most subacute and chronic progressive degenerative diseases of the central nervous system have been classified as disorders of unknown etiology. Few, if any, are curable and although some are genetically determined, many cases of the same disorders are sporadic and do not have a history in a close relative. That apparently heredofamilial forms of some progressive degenerative disorders would prove transmissible as their sporadic counterparts came as a surprise and awakened the possibility of a pathogenesis regulated by a genetically determined susceptibility, or a turning on, or derepression, of a usually masked or latent, potentially slow-acting virus.

Kuru proved the first chronic human neurologic disease to be a virus-induced slow infection. The demonstrated transmissibility of this disease set the field for intensive investigation of many more important degenerative diseases that we then realized might have related pathogenesis. Thus, soon after the transmission of kuru, the presenile dementias of the Creutzfeldt-Jakob type were proved to be equivalents of kuru, but with worldwide nonexotic distribution. Both the sporadic and familial forms of this type of dementia have now been shown to be transmissible to a wide variety of experimental hosts. We have recently demonstrated that the virus of CJD in human brain tissue is not inactivated following more than 8 months storage in 10% formal-saline fixative. The disease is transmissible from human to human by transplantation surgery and is associated with an incubation period of 18 months. Studies on the pathogenesis of the disease in human patients have shown that the virus reaches high concentration in brain and is widely distributed in many tissues of the patient, e.g. brain, liver, kidney, cornea, lymph nodes. The occurrence of this disease in a pediatric neurosurgeon suggests strongly the possibility of occupational hazards in the handling of tissues of CJD patients. The development of the disease in a recipient of corneal grafts

from a donor dying with CJD warrants grave concern over the utilization for transplantation of tissues from donors dying with any of the currently recognized presenile and senile dementias and sclerosing diseases.

Prior to our isolation of the virus of transmissible presenile dementia of the Creutzfeldt-Jakob type, the world literature contained references to about 150 cases of this disease. Since 1968 we have collected tissues at surgical biopsy or early autopsy from more than 250 additional cases and have records on many more suggesting an annual incidence of about 1-2 per million population. We have been aware of occasional clustering of cases in small populations, admittedly lacking in natural boundaries, and the unexplained absence of any cases over periods of many years in some large population centers where, at an earlier date, cases were more frequent. This geographic and temporal clustering does not apply to a majority of cases and is unexplained by the 10% of the cases that are familial. Matthews has recently made a similar observation in two clusters in England. There are two reports of conjugal disease in which husband and wife died of CJD within a few years of each other. The demonstration of a 30-fold higher incidence of CJ disease in Israel in Jews of origin from Libya (31.3 per million population) above that in those of European origin, may offer important epidemiological clues toward the elucidation of the source of infection. Although most cases of CJ disease are sporadic there are families in which the disease has occurred in siblings, parents and close maternal and/or paternal relatives over several generations. We have inoculated specimens from 11 familial cases. From 6 such families the virus has now been isolated by the experimental transmission of the disease to nonhuman primates of several species, demonstrating for the first time that in MAN disease with apparently genetic determination may be caused by a "slow virus". In addition to the familial cases we have transmitted CJD from 60 sporadic cases.

These findings have added impetus to our already extensive studies of Huntington's chorea, Pick's disease, Parkinsonism-dementia and even senile dementia. They have formed the basis of our most recent discovery that two cases of familial Alzheimer's disease were transmissible to nonhuman primates inducing a fatal subacute spongiform encephalopathy in the experimental host and associated with incubation periods of 23 and 29 months, respectively, which is not unlike those observed in animals dying with experimental CJD. It is worthy to note that the brain biopsy tissue of one of the patients that caused a fatal disease in the nonhuman primate is from a patient who is still alive and demented 4 years following neurosurgery. More recently we have observed a fatal subacute central nervous system disease in primates inoculated with brain tissue from a patient that had died with a diagnosis clinically and histologically of progressive supranuclear palsy. Thus, these investigations have so broadened the picture of CJ disease that we now must refer to transmissible virus dementias. This is necessary, since the diseases in patients with subacute or chronic dementia whose clinical and pathological diagnosis have been Alzheimer's disease, *papulosis atrophicans maligna* of Degos, and progressive supranuclear palsy have been transmitted to nonhuman primates as subacute spongiform encephalopathies. Thus, the trail from kuru to CJ disease now embraces studies of presenile and senile dementias of all sorts, including "dementia praecox", the organic brain disease associated with late uncontrolled schizophrenia.

The demonstration that non-inflammatory chronic degenerative, even hereditary, central nervous system disorders can be of virus etiology made possible the demonstration that subacute sclerosing panencephalitis of children and adolescents and progressive multifocal leucoencephalopathy were also "slow virus diseases". These models, namely that of defective virus infections with cell-to-cell spread of virus or replicating virus subunits, and that of long latent, masked or suppressed infection reactivated by immunosuppression have led to reconsideration of the possibility that slow and defective infection may underlie the pathogenesis of multiple sclerosis, amyotrophic lateral sclerosis, Parkinson's disease and many other degenerative CNS disorders.

The immunological investigation of antibody activities in the cerebrospinal fluid and serum of multiple sclerosis patients, particularly those with acute courses as in neuromyelitis optica or Devic's syndrome, has led to our suspicion that not all but at least some of these patients are suffering from delayed and slow, defective virus infections, probably with defective measles virus or with an as yet unidentified myxovirus antigenically related to measles. Intensive studies with whole virus and virus subunit antigens of serial CSF and sera, and γ -globulin extracted from autopsy brain specimens for antibody activity is underway in attempts to find clues as to the possible role of virus infection in this disease. Nucleic acid homology studies using MS and ALS brain tissue and purified viral nucleic acid probes made from selected candidate viruses are being pursued.

The seroepidemiological study of the JC papovavirus isolated from the brains of patients dying with PML has confirmed the hypothesis that this disease may be caused by a latent virus which infected man early in life. We have determined the presence and patterns of the acquisition of antibodies to JC, BK, and SV-40 papovaviruses in isolated primitive groups with no contact either with subhuman primates or with vaccines. This has helped elucidate the biological behavior of these viruses.

In an attempt to unmask the viruses in diseases suspected of being caused by defective or masked virus infection, we are studying brain cells and cells derived from visceral tissues that have been grown in vitro by both primary explant and trypsinized disruption techniques from human and nonhuman tissues obtained at surgical biopsy or early autopsy. Co-cultivation, cell fusion with sendai and lysolecithin, and shocking with BrdU and IrdU induction are major techniques employed. The presence of a virus in these cultures is sought by a search for cpe, hemadsorption, staining for inclusion bodies and fluorescent antibodies, interferon production, viral interference, exposure to cytotoxic antibody, and visualization of virus particles by electron microscopy.

As a byproduct to the work, many masked and latent viruses have been isolated from tissues obtained from chimpanzees and many species of old world and new world monkeys. These include herpesviruses, new ape and simian adenoviruses, a wide series of new simian foamy viruses and several strains of type C oncornaviruses from the brains of three gibbon apes. These three type C viruses (GBr-1, GBr-2 and GBr-3) were isolated by co-cultivation of normal gibbon ape brain tissues with cultured mammalian cell lines. The tissues,

frozen since 1968, were obtained from two animals inoculated with brain extracts from human patients with kuru and from one uninoculated cagemate. By viral interference tests and by immunologic studies of the viral polymerases and major internal structural proteins (P-30), the new isolates are typical members of a group of mammalian type C viruses infectious for primates. By nucleic acid hybridization, the viruses isolated from the gibbon brains, while highly related to one another, can be readily distinguished from the previously isolated type C viruses of this group. The infectious type C viruses isolated to date can now be classified into four distinct groups. A similar search for C particles in the human kuru brains that had been used to inoculate two of the gibbon apes, employing identical techniques, failed to elicit virus or reverse transcriptase.

Of particular interest has been the isolation of adenoviruses from the urine of chimpanzees. Viruria has persisted in 3 chimpanzees for over 16 months. Two animals died but neither had viruria at the time of death and cell cultures derived from the urinary tract of one animal grew normally without cytopathic effect. Affected chimpanzees shed more than 10^3 logs of virus in their urine. The virus has been shown to induce lethal tumors in hamsters. Studies of T-antigen associated with one of the new strains of chimpanzee adenovirus (A-226-1) are in progress.

New simian foamy viruses continue to emerge from our cultivation of nonhuman primate tissues in vitro. Although they are associated with a reverse transcriptase they have not as yet caused cell transformation in vitro nor have they induced tumors in vivo in monkeys or laboratory rodents. It has been shown that these syncytia forming viruses are present not only in brains but in all major organs, lymphocytes and placental tissues of nonhuman primates. In every instance tested homologous antibody is present in the animal from whose tissues the virus has been isolated. During this past year considerable emphasis has been placed on studies of the foamy viruses. This has been warranted not only because they pose a unique problem in their frequent detection in animal tissues without evidence of an associated disease, but more because of the reported isolation by Epstein of a human foamy virus from a patient with nasopharyngeal carcinoma and the isolation by Balayan in Entebbe of two additional syncytium forming viruses from Burkitt's lymphoma organ cultures. Their role in the etiology of disease is presently unknown but we have extensive studies underway to determine the significance of these viruses. We have recently completed a "cross" fluorescent-antibody study of the Epstein human foamy and simian foamy serotypes 1 thru 8 in an effort to determine if a serological relationship exists between the human isolate and the simian prototype viruses. Thus far, these studies show that using indirect and direct FA tests a strong two-way cross-relationship exists between simian foamy virus type 6 isolated from the chimpanzee and the human foamy virus. This has been confirmed by neutralization studies. No cross reactivity has been demonstrated to foamy viruses types 1, 2 or 3 by either foamy type 6 or the Epstein human foamy virus. Scores of human sera have been examined for antibody to the human virus but no positive sera have been found. Additional studies employing sera from East African patients with nasopharyngeal carcinoma and controls are being pursued. The data thus far strongly suggest that the Epstein foamy virus may be another strain of

chimpanzee type 6 foamy virus possible acquired through contact. Molecular hybridization studies to determine sequence of viral genomes shared by these two viruses are underway in collaboration with Todaro and his group at NCI. During this fiscal year we have isolated additional strains of foamy viruses from chimpanzees (4), cynomolgus (1), rhesus (1), and spider (1) monkeys. Of interest is the new spider isolate which appears to be a second serotype isolated from this species of new world monkey.

Thus, although a plethora of virus strains have been successfully unmasked in tissues from nonhuman primates grown in vitro the same techniques applied to human tissues in our search for latent, masked and incomplete viruses in human tissues, particularly of the central nervous system, have not proved rewarding. A total of 125 human tissue specimens were received in tissue culture fluids during this reporting period. Sixty-five of these were from autopsy or skin biopsy specimens from patients with ALS or ALS-PD on Guam and 60 were from patients dying with other diseases, e.g. CJD (4), astrocytomas (2), dementia of unknown etiology (2), multiple sclerosis (1), and glial sarcoma (1). More than 1000 ampules, each containing 10^6 to 10^7 viable cells of these established cell lines were prepared and stored in liquid nitrogen. Coverslip preparations of selected cultures were made for FA and acridine orange staining and some were stored at -70°C for future testing. Supernatant fluids of selected cultures were tested for viruses by subculturing onto primary HEK and WI-38 cells. No cytopathogenic agents were detected.

In one instance a very thorough search using viral interference and FA techniques was made to find rubella virus in the brain of a patient with MS and high specific rubella virus antibody titers. No agent was isolated and falling antibody titers to the rubella antigen suggested that the patient may simply have undergone an incidental preceding infection. In yet another instance, a radical left hemispherectomy was performed on an MS patient in Vienna, Austria and the biopsied tissues were submitted to our laboratory for study. A major effort is being made to isolate an agent from these tissues by direct animal inoculation and by in vitro studies. Co-cultivation of this brain upon receipt with 12 established cell lines of various type tissues has yielded no recognizable cpe, no evidence of hemagglutination (tested at passage levels 2 and 4), and no evidence of reverse transcriptase. Lysolecithin fusion and low temperature fusion of the patients brain cell line and normal human brain with MA-184, MA-134 and MA-104 cells has yielded no recognizable cpe or hemagglutinin. BrdU and IrdU treatment of the MS and normal human brain cultures co-cultivated with MA-184, MA-134, and MA-104 cell lines showed no reverse transcriptase or HA activity. HA activity was not detected in allantoic fluids harvested on day 13 from embryonated hens' eggs inoculated on day 8 with fused MS and normal human brain cultures. Further passages of allantoic fluids in embryonated eggs is underway and all specimens are being tested for HA activity. The diagnosis of MS made by the contributing physician was confirmed in the US on gluteraldehyde fixed brain tissue which contained new active plaques and evidence of old plaques. Some of this material has been made available under code for testing for the presence of the putative MS virus from Henle in Philadelphia.

The long maintenance of brain cells, presumably astroglial cells, dedifferentiated on passage to resemble fibroblasts, regularly result in cell lines which propagate for very long periods or indefinitely and show many properties that suggest transformation. We have been impressed at the better and quicker growth of brain cell extract cultures from subjects with subacute spongiform virus encephalopathies than from accident victims and normal animals. The more obvious transformation, with full loss of contact inhibition among cells, has occurred in three cases with human brain cells and two cases of mouse brain cells, one derived from normal mice and the other from scrapie-infected mice, respectively. In the first instance of human brain cell transformation, a CJ disease patient's brain derived cell line spontaneously transformed at the third serial subculture level and was subsequently shown to be carrying a Mason-Pfizer monkey mammary tumor virus related agent (MP-MV oncornavirus). The "C" type particle isolated from these transformed cells is weakly associated with a reverse transcriptase and by molecular hybridization studies has been shown to be closely related but not identical to MP-MV. The validity of this isolate from human brain has been supported by the work of other investigators who have, subsequent to our report, isolated similar type MP-MV related viruses from human tissues. The other two transformations we have observed have occurred in two brain cell lines from CJ patients which showed full loss of contact inhibition but without the presence of recognizable virions in the transformed cells. In addition, a transformed cell line from a class II astrocytoma has been established and carried for many passages suggesting infinite duration; however, we have not been able to isolate an agent. The cultures from this astrocytoma do not manifest a reverse transcriptase either on their own or following co-cultivation on "C" type virus permissive cell lines, and they do not induce tumors or clinical disease in animals. Finally, it is significant to note that many of our cell lines derived from central nervous system and other types of tissues from humans and experimentally inoculated and control animals appear to have unexpectedly long life, suggestive of transformed cells. Studies are underway to determine the state of these cell lines; they are being routinely monitored for evidence of reverse transcriptase. Thus, these apparent transformations and the occasional sudden spontaneous complete transformation of human brain cell lines continue to stimulate speculation that the slow virus-cell interaction in the degenerative central nervous system diseases may be related to the slow virus-cell interaction in cell transformation and carcinogenesis. The use of the term "temperate" in naming our laboratory in 1962 seems to have been prophetic.

The remarkable demonstration that subacute fatal brain diseases are transmissible highly pathogenic diseases has overshadowed the full appreciation that they are caused by a group of "atypical" and "unconventional" viruses unlike any others known to physicians and microbiologists in the field of infectious diseases, which we have termed the subacute spongiform virus encephalopathies. Other central nervous system degenerative diseases are slow, rumbling, non-productive even defective infections caused by viruses such as measles, rubella, cytomegalovirus, herpes simplex, adenovirus type 32, and true encephalitis viruses. The "atypical" viruses (kuru, Creutzfeldt-Jakob disease, familial Alzheimer's disease, scrapie and transmissible mink encephalopathy) are serially transmissible, self-replicating agents that pass through membrane filters which withhold all bacterial-sized microorganisms.

They cause a fatal disease reproducible with only slight variations noted in the incubation periods, clinical signs and neuropathological lesions they induce in a wide variety of experimental hosts. The "atypical" viruses (kuru, CJD, scrapie and TME) have unusual resistance to ultraviolet radiation and ionizing radiation, to ultrasonication, to heat, proteases and nucleases and to formaldehyde. They are not associated with a recognizable virion on electron microscopic study of infected cells *in vivo* or *in vitro*, or concentrated virus preparations by zonal sucrose density banding.

The atypical properties of scrapie have led to speculations that scrapie lacks nucleic acid and may be a "self-replicating" membrane fragment. Our data have demonstrated that scrapie infectivity is intimately associated with the plasma membrane of infected cells, but our working hypothesis on the nature of scrapie includes nucleic acid of small molecular weight. Thus, a major effort in this laboratory has been and continues to be directed toward the molecular-biological elucidation of the composition, structure and interrelationships of this group of atypical viruses. On the molecular level two approaches to the *in vitro* monitoring of picogram quantities of scrapie specific nucleic acid extracted from infected brain are being tested. The first is nonenzymatic labeling with ^{125}I by a modified Commerforal procedure. The second is long term exposure of ethidium fluorescence by utilizing slab gel electrophoresis in direct comparison of nucleic acid extracts.

Scrapie virus has been partially purified by fluorocarbon precipitation of proteins and density gradient banding using the zonal rotor technique. Low-speed supernatants of pressure-disrupted scrapie infected mouse brains were fractionated either directly or after differential ultrafiltration by isopycnic centrifugation in buffered sucrose gradients. In the former experiment, 86% of the recovered scrapie infectivity was concentrated in three peaks of densities, 1.19, 1.26, and 1.29 grams per ml. In the later experiment, after ultrafiltration, 87% of the recovered infectivity was distributed heterogeneously from density 1.14 to 1.24 grams per ml. Although the infectivity regions contained considerable UV 260 and 280 nm absorbing material, the absorbance profiles did not suggest any obvious correlation with the infectivity peaks. The three lysosomal (N-acetyl- β -D-glucosaminidase, acid phosphatase, β -galactosidase) and one mitochondrial (INT-succinate reductase) enzyme activities assayed banded at lower densities than the infectivity peaks. Electron microscopy revealed smooth vesicular membranes and mitochondrial fragments in all regions containing high infectivity. In another series of similar experiments on the purification and concentration of scrapie, infected mouse brain suspensions were processed through cell disruption by pressure bomb, centrifuged, filtered and sonicated to produce a "cleaner" preparation of infected starting materials with which to perform density gradients, simultaneously, on CsCl, sucrose, and metrizamide. The goal was to determine whether the density of the infective particle is influenced by physical characteristics of the gradient material. Preliminary results indicate that freeze-thaw cycles do not affect the distribution of infectivity although they markedly affect the distribution of protein and nucleic acid in the gradient. Further, infectivity is well preserved in CsCl over the time limit tested and the distribution of infectivity is the different gradient materials is similar, with a broad peak at about a density of 1.20 grams per ml.

We have continued our work in collaboration with Dr. Raymond Latarjet on inactivation of scrapie, kuru and CJD viruses by UV irradiation and ionizing irradiation. The results of these studies confirm the reported high resistance of scrapie virus to UV inactivation at 250 nm and an UV inactivation action spectrum with 6-fold increased sensitivity at 237 nm over that at 254 nm or 280 nm. This may not be taken as proof that no genetic information exists in the scrapie virus as DNA or RNA molecules since work with the smallest viruses, called viroids, indicates a similar resistance to UV inactivation in crude infected plant sap preparations. There is also a great effect of small RNA size on UV sensitivity, as has been shown by the high resistance of the purified viroid RNA to UV inactivation, and a similar high UV resistance with the purified very small RNA (80-100,000 daltons MW) of tobacco ring spot satellite virus. Partial purification of scrapie infected mouse brain with fluorocarbon selective deproteinization techniques only slightly increases UV sensitivity at 254 nm. Fluorocarbon purified scrapie was not inactivated by RNAase A, RNAase III or DNAase I. Autoclaving (212°C/20 lbs psi for 45-60 mins), dry heat sterilization procedures (289°C/2 hrs) and treatment with 5% sodium hypochlorite completely inactivated scrapie virus both in whole tissues and in suspensions of infected tissues.

Working with Cho (Nature 257: 685-686, 1975) we have been able to confirm the presence of 12-14 nm size particles in normal and scrapie infected mouse brains, and to extend these observations to normal and kuru and CJD-infected chimpanzee brains which have undergone extensive extractions with fluorocarbon followed by ultracentrifugation concentration. We have been unable, however, to distinguish infected from uninfected tissue using Cho's method. We have observed both the Cho-reported "infection specific band" from sedimentation to equilibrium as well as "clear" 14 nm particles in both infected and uninfected tissue. These same empty particles are remarkably similar in morphology, size and density to those observed in purified preparations of horse ferritin. However, due to the importance of Cho's claim to have isolated the scrapie virus, we will continue to characterize the particles obtained from both infected and uninfected tissues, by immunological and biochemical methods in order to establish their identities more definitively. Our data strongly suggest that the 12-14 nm particles are apoferritin particles and not the viruses of scrapie, kuru or Creutzfeldt-Jakob disease.

In the previous annual report we gave preliminary data on the use of freeze-fracture studies of cell membranes of scrapie-affected mouse cerebellum. These studies had been initiated in the hope of better defining the membrane subunit structures we assume contain the infectious genome of scrapie. During this year these studies to elicit structural changes in the membrane of cells infected with scrapie were continued. Control mice and mice inoculated with scrapie virus, matched as to source, strain and age, were perfused with aldehyde fixatives through the heart, during the asymptomatic incubation period and in various stages of clinical disease. Fixed brain slices and pellets were frozen in glycerol and freeze fractured in a Balzer freeze-etcher apparatus. Replicas of the fractured membrane were then studied under EM. Structural changes were observed in the membranes of cells from scrapie-infected mice but not in the brains from normal control mice. In the scrapie brain abnormal changes were observed in the neural and astrocytic

membranes of clinically-affected mice. The inner most limiting membrane of vacuole walls lacked the intramembrane particles present on normal neuronal and glial membranes. Astrocytes around the vacuoles were characterized by an increased number of "assemblies". Structural abnormalities were also observed in membranes at some distance from the vacuoles. The unusual fracturing pattern observed suggested that abnormal adherence might exist between cells at a point where infection is spreading from cell to cell. The freeze-fracture technique failed to demonstrate a scrapie specific virus-like structure.

Preliminary studies already reveal the same type of structural changes of membrane in brain cells from CJD infected tissues. Attempts are in progress to characterize further the vacuoles of status spongiosis in both human (original) and nonhuman primate (transmitted) CJ diseases by correlating the findings of transmission (TEM) and scanning (SEM) electron microscopy. Both the biopsied and autopsied brain specimens from two CJD patients and two CJD monkeys (1 spider and 1 capuchin) were prefixed in gluteraldehyde. The monkeys were killed by Karnovsky's perfusion method. All tissues were post-osmicated for both TEM and SEM. For SEM, the fixed specimens were placed in acetone, freeze-fractured in liquid nitrogen, and then critical-point dried before cooling with gold for SEM. Both human and monkey specimens were processed in the same manner along with two human controls and a normal monkey. Vacuoles were readily detected on both TEM and SEM and appeared to be composed of clusters of blisters of varied sizes. On SEM, these vacuoles contained ruptured or intact physaliphorous blisters of which inner and outer surfaces are attached with small vesicles (70 to 150 nm) and particles. The outer surfaces of the blisters are uneven and often demonstrate a superficially ulcerated appearance in comparison to the smooth fractured surfaces in the vicinity. On TEM, many small vesicles within the blisters suggest that at least some of the blisters are derived from swollen terminal axons. The membranes of the blisters are irregularly curled and fragmented and often appear puffy and amorphous. They are associated with fine dense granules (5 to 10 nm) in chains resembling staining precipitates. This puffy membrane may not be a tangentially cut unit membrane, but an altered and focally thickened membrane. The features of the vacuoles, on either TEM or SEM, in the human and monkey CJ diseases are essentially identical and no similar changes were observed in control human and monkey brains examined. Too little is known about the appearance of various pathological states in freeze-fractured neural membranes to determine whether any of these changes are specific to scrapie and CJ disease. They are changes absent in normal tissues examined and may represent changes in the organizations of proteins associated with the development of virus infectivity in membrane subunits.

In an attempt to develop in vitro method of studying scrapie, kuru and CJD we have prepared SV-40 virus-infected cultures from brains of chimpanzees with experimental kuru and CJ disease, brain cell lines derived from each of two patients with CJ disease, and mice and hamsters with scrapie disease. These cultures appear to be altered in properties so that they grow faster than the original cultures (transformed), and all express SV-40 T-antigens. Animals have been inoculated with aliquots of these cultures to determine if the agents of spongiform encephalopathy persist and/or replicate in these

transformed cell lines. If virus replication continues in such cells with rapid cell division rate we would have a much needed tool for scrapie work both for providing known scrapie infected cells and for production of large quantities of infectious virus.

To investigate the intracellular location of the scrapie virus by the techniques of cell enucleation and cytoplasm fusion we have derived a mutant brain cell using one of the scrapie mouse brain cultures infected with SV-40 virus, and selecting clones of cells highly resistant to ordinarily lethal doses of chloramphenicol. Simultaneous resistance to amphotericin-B demonstrates that this is not due to altered permeability; chloramphenicol resistance of this type is known to be carried by mitochondrial genes.

We have continued our work on the host range of the subacute spongiform virus encephalopathies. The cat is susceptible to CJ disease; the mink is susceptible to kuru. We expect that these two non-primate hosts for the human virus infections will make further study of these viruses somewhat less expensive. We are trying to confirm the reports of the transmission of CJ disease to mice and to guinea pigs; in our earlier work these species have not been susceptible.

The transmission and serial passage of scrapie virus from American scrapied sheep brain, as well as from goats and mice infected experimentally, to several species of new world and old world monkeys, with disease clinically and pathologically indistinguishable from that produced by CJ disease in the experimental primates, has reawakened our earlier suspicions that scrapie may be closely related to the occurrence of the subacute spongiform virus encephalopathies in man. In these studies we have demonstrated evidence of virus-strain variation. Passage of the English strain of scrapie (Compton) through nonhuman primates has altered its properties so that it has not yet induced disease following inoculation of scrapie-infected monkey brain into sheep, goats or several mouse lines known to be susceptible. In contrast, passage of American strains of scrapie through nonhuman primates has not altered their host range and inoculation of these materials into sheep, goats and mice results in clinically and histologically confirmed scrapie. Thus, the biological property of host range changes on passage in new hosts as do those of incubation period and virulence. Such properties cannot be used to establish relationships between the atypical viruses of this group. In the absence, as yet, of proven antigenicity or identified nucleic acid in the agents, neither serological specificity nor nucleic acid homology can be used to answer the compelling question of the relationship between the viruses of kuru, Creutzfeldt-Jakob disease, scrapie and transmissible mink encephalopathy.

The NINCDS Research Center, located on Guam, for studying Amyotrophic Lateral Sclerosis and parkinsonism-dementia among the Chamorro people on Guam and in the Mariana Islands has been transferred to the scientific supervision of this laboratory. In view of over two decades of fruitless search for the etiology of either of these syndromes we have now done some hard thinking about the future course of studies. For ALS-PD there are not the enticing epidemiological data found in multiple sclerosis which suggest strongly a foreshadowing event in childhood which determines the likelihood of MS in

later life, as the immunological finding of high measles active antibodies in the CSF and serum, and of γ -globulin changes suggestive of an infection-precipitated immunological event. The extensive primate and small animal and tissue culture inoculation studies on brain biopsy and early autopsy tissue have all been negative. We have transferred part of our tissue culture facility to the Guam laboratory and collected brain and visceral tissues from early autopsies on ALS and PD and control patients to establish over 60 cell lines of brain and peripheral tissues for biochemical and genetic and microbiological study back at NIH and in collaborating laboratories abroad. Also, we have established fibroblast cultures from many patients from skin biopsies for similar studies. These have been successfully carried to the NIH and to other laboratories. Extensive attempts to obtain a oncornavirus from such cultures by co-cultivation with a "walgurnisnacht-like" array of reputedly C-type virus-free cells (bat lung, dog thymus, gorilla brain, human rhabdomyosarcoma, mink lung, rhesus lung, cat kidney, wild mouse) used by Todaro in the National Cancer Institute's screening program with monitoring for the appearance of reverse transcriptase activity of C-type particles and electron microscope screening has yielded no agents in spite of the usual success of such techniques when we use brain tissue from chimpanzees, gibbons or monkeys (see above). Biochemical and genetic analyses are in progress and the aliquots of the live cells remain available in liquid nitrogen storage.

Viola and Brody have previously reported that a DNA polymerase capable of copying a RNA template was present in extremely low concentrations in brains from Guamanians with ALS-PD. Only reactions using brains from Guamanian patients and controls generated hydrogen bonded RNA-DNA hybrids, whereas a number of controls and ALS patients from the United States with other neurological diseases were negative. This "suggested" RNA instructed DNA polymerase (RIDP) in Guamanian brains and not a normal cellular RNA-primed DNA-dependent DNA polymerase. However, because these results were obtained from relatively crude preparations of brain, in collaboration with Dr. Viola, we begin a systematic fractionation and partial purification of the relevant DNA-polymerase from normal brain and a comparison of these with DNA-polymerase in ALS-PD brains to ascertain if a viral polymerase is present in the diseased tissues. The long term goal of these studies is the unambiguous characterization of viral RIDP in ALS-PD brains IF present, and the development of specific antisera to this enzyme. During the first nine months of this study significant accomplishments have been (1) development of the methodology for DNA-polymerase purification from human brain; (2) partial purification and characterization of two enzymes from normal brain not previously detected in brain from any species; (3) systematic fractionation of normal human brain and assay for endogenous RIDP activity with failure to detect this enzyme in such normal control brain; (4) detection of an end-addition enzyme (terminal deoxynucleotidyl transferase) in normal brain of potential general importance in brain biology; and (5) large-scale purification of the DNA polymerase in brains of Guamanian ALS-PD patients, designed to detect and purify any RIDP present. Work has also continued on the application and development of nucleic acid hybridization techniques for the detection of viral nucleotide sequences in DNA extracted from diseased central nervous system tissues and cells in vitro. Preparations of CNS DNA from Chamorro patients with ALS-PD

have, within the limitations of the methods employed, been negative with purified viral probes of poliovirus type 1, HSV-I, HSV-II, murine sarcoma virus and Rauscher leukemia virus. The principal obstacles in these studies have been difficulties in the preparation of pure viral probes of high specific activity, and problems in the extraction of nucleic acids from frozen tissues. Nevertheless, large quantities of unlabeled polio 1, polio 2, polio 3, HSV-I, HSV-II, visna virus and 1504 virus (neuropathic wild mouse oncornavirus) have been prepared, concentrated and will be used in the preparation of radioisotopically-labeled probes.

This year other studies on Guam have included HLA typing, determining immunoglobulin levels, cellular immunity as measured by both in vitro and in vivo methods and establishment of extensive tissue culture work involving diseased and control tissues. HLA typing was performed on 60 PD patients, 23 ALS patients and 67 controls. A higher percentage of W-16 (HLB-W39) a second locus antigen was seen in the PD group (25%), than in the ALS group (15%) and in the controls (7%); these differences are not statistically significant. The population studied showed a distribution of antigens commonly associated with Oriental populations with a high preponderance of HLA 2, 9, 10, W-10 and W-5. HLA 3 and 7 were detected in only one ALS case and one control. Immunoglobulin levels were measured in 57 normal Guamanians, 34 ALS patients and 61 PD patients. IgA levels were significantly higher in PD patients than in controls (255 ± 11.7 IU/ml vs 223 ± 8.9 IU/ml $p < 0.05$) while IgM levels were significantly lower than control PD (162 ± 9.7 IU/ml vs 200 ± 12.4 IU/ml $p < 0.05$). ALS cases generally showed slightly higher levels of all three immunoglobulins than controls. Diminished cellular immunity as measured by both in vitro and in vivo parameters exists in both ALS and PD patients. Enumeration of T-cells by the Rosette method showed that both PD and ALS patients were significantly lower than age-matched controls both by the percentage of circulating lymphocytes that carried the marker and total number of circulating T-cells. (ALS $\bar{x} = 970 \pm 41.0$ $p < 0.05$; PD $\bar{x} = 866 \pm 80.0$ $p < 0.05$; normals $\bar{x} = 1570 \pm 70.1$ $p < 0.05$). Skin testing of both groups showed that they were less reactive to three standard skin test antigens: PPD, candida extract, and streptokinase-streptodornase. The rosette totals correlate with the skin test reactivity, in that those patients with the low numbers of T-cells were least reactive on skin testing.

Our discovery of an even more intense high incidence ALS focus (although smaller) in Jaqai and Auyu peoples of West New Guinea and the finding of parkinsonism and dementia syndromes in high incidence in this totally independent, genetically unrelated focus is further evidence that a common etiology underlies the different clinical and pathological syndromes. These foci of high incidence of ALS-PD have now been found in three Pacific populations namely, the Kii Peninsula of Japan, southern West New Guinea, and on Guam, Rota, Tinian and Saipan. These populations obviously continue to represent potential but unsolved keys to the solution of the ALS enigma.

We have continued our previously reported studies on focal movement disorder in rhesus monkeys following their experimental infection with a strain of tick-borne encephalitis. Using the PK-15 cell-Langat virus plaque system we examined neutralizing antibodies in the sera of monkeys recovering

from experimental tick-borne encephalitis. No difference in antibody levels was seen between monkeys with and without chronic movement disorders. Titers were low, presumably because Langat virus was used instead of the homologous strain of virus.

In yet another series of experiments we have been studying persistent asymptomatic cytomegalovirus infections in monkeys. CMV antigens were prepared for evaluating cell-mediated immunity (CMI) using the lymphocyte transformation test, with incorporation of tritiated thymidine into TCA-precipitable material as evidence of transformation. Preliminary tests, using both live virus and UV-inactivated antigens, suggest that monkeys chronically infected with CMV have persistently positive lymphocyte transformation in the presence of homologous simian, but not with the antigenically related heterologous simian or human CMV's. The same seems to be true of humans. One vervet monkey inoculated with rhesus CMV showed no evidence of acquired CMI to the rhesus virus. Further immunization studies with monkey and human CMV's are in progress. Finally, although we have never isolated CMV from chimpanzee urine, we have detected antibodies in chimpanzee sera which react with human and simian CMV antigens by FA.

Thus, the studies we have been conducting have provided new insights into our understanding of the etiologies of chronic diseases of the human brain and have been instrumental in providing additional models for studying the basic mechanisms of persistent, latent, masked and defective viruses. The most significant unanswered crucial questions arising from our studies on the subacute spongiform virus encephalopathies are related to the biological origin and mode of survival of the agents in nature and the relationship of the transmissible agents to each other. The diseases these viruses evoke are not artificial diseases, produced by researchers tampering with cellular macromolecular structures, as some would have it. They are naturally occurring diseases, for none of which do we know the mode of dissemination or maintenance which is adequate to explain their long-term persistence. Fore kuru, caused by the contamination of close kinsmen within a mourning family group by the opening of the skull of dead victims in a rite of cannibalism, during which all girls, women, babe-in-arms, and toddlers of the kuru victim's family were thoroughly contaminated with the virus, seems to provide a full explanation of the unique epidemiological findings in kuru and their change over the past two decades. The disease is gradually disappearing with the cessation of cannibalism and has already disappeared in children, with progressively increasing age of the youngest victim. However, this does not provide us with a satisfactory explanation for the origin of kuru. Was it the unlikely event of a sporadic case of worldwide CJD, which in the unusual cultural setting of New Guinea produced a unique epidemic? Serial passage of brain in man in successive cannibalistic rituals might have resulted in a change in the clinical picture of the disease, with modification of the virulence of the original agent. Another possibility might be that the serial brain passage that occurred in this ritual inoculation of brain from successive victims in multiple sequential passages into their kinsmen yielded a new neurotrophic strain of virus from some well-known virus. We must also wonder if a ubiquitous, or at least a well-known virus may not be modified into a defective, incomplete, or highly integrated or repressed agent in vivo in the course of its long masked state in the individual host. Such a

modified virus may no longer be easily recognizable either antigenically or structurally because of failure of full synthesis of viral subunits or of their assembly into a known virion.

The successful transmissions of scrapie to five species of nonhuman primates in which the virus induces a disease clinically and neuropathologically indistinguishable from experimental CJD provides an additional explanation for the origin of human spongiform encephalopathies. Certain strains of the scrapie virus, as a result of passage into nonhuman primates, develop an altered host range, for they no longer produce disease when inoculated back into sheep, goats or mice. Our data make it clear that neither the incubation periods nor host range, nor the distribution or intensity of neuropathological lesions, can be interpreted as having any significance toward unraveling the possible relationships of the four viruses causing the subacute spongiform virus encephalopathies. The possibility that all four of these viruses are not just closely related agents, but different strains of a single virus which have been modified in different hosts, is easily entertained. It is the unraveling of this problem that remains the thrust of these studies.

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ANNUAL REPORT

July 1, 1975 through June 30, 1976
Clinical Neurosciences Branch
National Institute of Neurological and Communicative
Disorders and Stroke

Cosimo Ajmone Marsan, M.D., Chief

Summary of Program Activity

This is the first Annual Report of this Branch which has been created recently as an amalgamation of the pre-existing Branch of Electroencephalography and Clinical Neurophysiology with part of the old Neurosurgical Branch. The new Branch consists of two sections: Functional Neurosurgery and Clinical Neurophysiology, the activities of the latter including, as in the past, a clinical-diagnostic service for all patients in the Clinical Center. In the overall evaluation of the activities of the Branch, and in particular of those of the Section on Functional Neurosurgery, it should be pointed out that these have been hindered by the absence of this section's chief (Sabbatical leave) as well as by the consequent unavailability of new clinical material through the entire fiscal period.

1. Clinical-Diagnostic Service

The activity related to this Service in the just completed fiscal period has involved a total of 3.8 man/years (0.8 professional and 3.0 technical-secretarial). This total, which is similar to that of the preceding fiscal period, is down from the over 5 man/years of recent years. The difference is due to the reduction in technical staff (2 EEG technologists versus 3-4 in the past). Whereas this reduction has not significantly affected the total number of EEG examinations, it has seriously interfered with the handling of emergency referrals, frequently requiring the cancellation or rescheduling of examinations (of particular inconvenience in the case of out patients) and limiting the service to the week-day's working hours. The occasional, unavoidable absence of one or both of the two technicians has, of course, accentuated these problems.

From the time the last Report was prepared (April 10, 1975) to that of the present Report (April 12, 1976) a total of 1310 EEG tracings have been obtained and interpreted, in patients referred to our laboratory as part of their routine clinical work-up or for specific research projects originating outside of this Branch and/or Institute. The distribution of these referrals according to the various Institutes of origin has been:

<u>INSTITUTE</u>	<u>NO.</u>	<u>%</u>
NINCDS (OP 358)	864	65.7
NIMH	196	15.0
NCI	107	8.2
NIAMD	62	4.8
NHLI	42	3.2
NIAID	28	2.1
NCHD	2	0.2
Misc.	9	0.8
TOTAL	1310	100.0

A considerable number of the 446 examinations requested by Institutes other than NINCDS had to be carried out on the ward, at the patient's bed or in the Intensive Care Unit. In addition to the EEG examinations, which include those performed in four patients with chronically implanted depth electrodes, three records were obtained directly from the exposed cortex in the course of surgical intervention. This unusually small number of non-routine procedures reflects the situation referred to in the introduction.

2. Research Activity

The present Report includes a total of 20 projects, 9 new and 11 representing a continuation of old projects. Of these, 7 are of a primary clinical nature while the other 13 are experimental. Two additional projects in which our professional staff have been actively involved are described in the Report of the Laboratory of Neural Control. Of the 20 projects, six have been completed while the others are expected to continue through or be completed within the coming fiscal period. A total of 14, related papers have been published or are currently in press.

a) Clinical

Work in the Functional Neurosurgery program has continued to provide new information of a fundamental nature regarding the human nervous system. Quantitative determinations of the density of Purkinje cells in the cerebellum of 7 epileptic patients were compared with those in 5 patients without epilepsy or neurologic disease. The controls showed a mean density of 3.56 Purkinje cells/linear mm. of cortex/10 μ section (SD = 0.18) while the epileptics had a mean density of 1.35 (SD = 0.82) cells. In all cases the loss of cells was accompanied by isomorphic gliosis. Three of the epileptics had devices for cerebellar stimulation implanted. Of these the patient showing the lowest count of Purkinje cells appeared to have the best degree of seizure control associated with stimulation. This curious situation raises question as to the role of Purkinje discharge in seizure control.

In fact, in another related study, carried out in epileptic patients receiving chronic cerebellar stimulation, it was shown that such a procedure (either unilateral alternating or bilateral) induces significant elevations in the levels of norepinephrine in their cerebrospinal fluid. These alterations in norepinephrine metabolism may be related to the seizure-suppression effect of cerebellar stimulation. In the meantime, a continuous follow-up study of the same patients with an effective method of double-blind stimulation would seem to indicate that in most subjects their seizure frequency tends to increase during periods of ineffective cerebellar stimulation. In spite of the fact that there has been no complete abolition of seizures, chronic stimulation appears to have unquestionable beneficial effects: the dosages of medical treatment has been reduced and the families of all patients are enthusiastic about the results since the patients have less frequent seizures and are more manageable and alert.

As part of the same project, two additional studies deal with the diagnosis and treatment of complex cases of medically intractable seizure disorders. Specifically these studies emphasize the use of chronically implanted recording electrodes and the information they provide for the localization of the epileptogenic process in patients with complex partial seizures and multiple or bitemporal "foci" and in patients with partial seizure originating in extratemporal structures. These and the preceding studies have been completed and related papers are either in press or have been published.

Another research project investigates the clinical ictal patterns in epileptic patients with electrographic evidence of epileptiform activity within the temporal areas. These seizures are rather complex and quite variable; the automatisms and vegetative symptoms predominate but motor patterns of both tonic and clonic nature are also observed. The availability of a relatively large number of carefully selected patients with a long follow-up and repeated EEG examinations offers an unparalleled opportunity to evaluate all their possible ictal patterns in detail. The findings, beside increasing our knowledge on the pathophysiology of "temporal lobe" seizures, complement those obtained in other groups of epileptics in the recent past and should prove of practical usefulness in the localizing diagnosis and classification of seizure disorders.

The opportunity offered by cortical exposure in the course of surgery for treatment of epilepsy has been utilized to study differences in oxidative responses to electrical stimuli in presumably pathological human cortex. This investigation based on the use of television fluorometry to monitor NADH changes, is expected to continue. Preliminary results and technical details have been published or are being prepared for publication. A summary is available in the description of the related project (01939b-05).

Another project deals with dyskinetic patients. In 5 of such cases undergoing thalamotomy through a frontal approach control radioenzymatic assays of lumbar CSF norepinephrine concentrations before and 12 days after surgery showed no significant alterations. Stimulation within the caudate nucleus, however, was followed by a significant fall in CSF norepinephrine concentration. This suggests that in man there are efferent noradrenergic pathways which are inhibited by stimulation in the caudate nucleus.

Clinical investigations were designed to identify brain mechanisms in man which mediate speech, language and memory. Gentle stimulation of the surface and depths of the human brain was used to plot distinct areas supporting linguistic behavior. In the posterior region, the vital language zone codes man's experiences and provides a mechanism for retrieving memory data. More anterior areas along the temporal lobe are not important in language and, instead, advance memory and learning by storing information. In the frontal region, there lies an area which is vital to the function of forming and uttering speech and articulation. In addition, this brain region regulates the rules of grammar and syntax in speech.

The subcortical relays to these areas, specifically the pulvinar nuclei, play a lesser role in language and memory. These nuclei enter stimulus processing at an early, pre-perceptual phase. Other subcortical systems like the cingulum do not influence cognitive processes directly, and seem to participate in directing behavior via emotional impetus to reinforce learning. In the right brain, these structures subserve different functions. In contrast to the verbal skills of the left hemisphere, the right hemisphere and subcortex employ visual, spatial strategies.

Brain-behavior relations in personality development and character formation were examined in terms of changes produced by different brain lesions. In a study of epileptic patients, individuals with left temporal insult displayed a poor self-image, tended to exaggerate their problems and to adopt a reflective, ideative posture. The right temporal epileptic patients were more self-assured, tended to deny personal problems and were described as very emotive.

In contrast to these studies where brain lesions were associated with intellectual disorders, "functional insult" via sensory deprivation had a disabling effect on neural development. Preliminary study of deaf individuals by non-invasive techniques revealed that the language brain under these conditions is less efficient in processing verbal information. These data argue for the reliance of language on auditory signals and the importance of cross-modal integration in language formation.

b) Experimental Research

A number of investigations deal with cortical metabolic changes in various experimental situations. Specifically they deal with the oxygen consumption, monitoring of blood flow, Hgb saturation and NADH fluorescence changes as well as with the release of potassium and its clearance in the cortex of different animals during and following local cortical stimulation, stimulation of the brain stem reticular formation, in the course of Metrazol-induced seizures and after the experimental production of chronic epileptogenic foci, as well as under normal or hypotensive conditions or during cerebral ischemia of various degrees.

The main purpose of one project was to confirm the validity of the cortical fluorescence method in vivo as an indicator of NADH changes and, indirectly, of changes in oxygen consumption rate. Following repetitive stimulation of a cortical region drained by the sagittal sinus, NADH fluorescence levels were monitored and oxygen consumption rate was calculated from the sinus blood flow and Hgb saturation values. The high, significant correlations found between the maximal changes in these two parameters (as well as between cortical $[K^+]_o$ changes and oxygen consumption) would suggest that, indeed, cortical fluorescence changes can be considered as a reliable indication of changes in oxygen consumption rate, even in the living animal. The practical application of the NADH fluorescence method in vivo and its reliable interpretation, however, have been limited in the past to relatively discrete or quasi motionless cortical regions because of the unpredictable changes of specularly reflected ultraviolet light with tissue motion and gross vascular changes. The introduction in our laboratory, of i.v. Na fluorescein, has provided a method to evaluate changes in the sagittal sinus blood flow (since such changes seem to be related to the product of the blood pressure and the square of changes in 529 nM fluorescence) as well as serving as a practical reference for NADH fluorometry. This has permitted the application of the latter method to the study of large areas of cortical tissue and in the presence of significant blood flow alterations. It has thus been possible to investigate metabolic changes during Metrazol induced seizures and reticular system activation. These investigations have shown that, in both situations, NADH tends to decrease (i.e. oxidation increases) over large cortical areas, but the occurrence and extent of NADH changes are quite variable in different and more discrete cortical regions (as indicated by densitometric analysis during videotape playback). This variability was particularly prominent during reticular activation which was generally accompanied by marked increases in blood pressure. Indeed, these experiments have shown the important role of blood pressure levels (and, more specifically, of cerebral ischemia) in determining the entity and direction of metabolic changes in these experimental situations. Thus, Metrazol-induced seizures in the normotensive animal were accompanied by a

parallel NADH decrease and cortical oxygen consumption increase, whereas the correlation between these two variables was lost during "hypotensive" seizures. In the same series of experiments it was also found that ischemia would tend to slow down the clearance of extracellular K^+ after the end of each ictal episode, suggesting the role of an O_2 -dependent process in the control of elevated $[K^+]_o$. Probably of greater interest, it was also noted that such clearance in both normotensive and ischemic preparations was not a monoexponential function. Such observation has been interpreted by implying a possible role of glial cells in this K^+ reuptake process. A similar interpretation has been supported by a different experimental approach. In fact, within a focus of epileptogenic tissue (glial scar induced by intracortical injection of alumina cream), a small but significant negative correlation could be demonstrated between extent of reactive gliosis and speed of extracellular K^+ clearance. On the basis of these two series of experiments and of related projects described in the previous Annual Report, the clearance of elevated $[K^+]_o$ in cerebral cortex would seem to depend primarily on active process. Further investigation of this question by means of a mathematical model developed from a set of experimental data would confirm this interpretation and indicate that passive diffusion is unlikely to contribute significantly to the clearance of elevated $[K^+]_o$ in the cortex. A final series of investigations which utilize the same general techniques was carried out with the purpose of studying NADH fluorescence changes in the intact, exposed myocardium during acute occlusion of a coronary artery in the cat. Such a procedure consistently caused an increase in NADH in the ischemic areas. As in the above mentioned experiments carried out at the cortical level, NADH fluorescence was evaluated in reference to Na fluorescein fluorescence. Of practical interest was the observation that an i.v. injection of fluorescein would result in the immediate, accurate delineation of the ischemic myocardial area which stood out as a dark region against the brightly fluorescent surrounding tissue. This technique could be useful in human coronary vascular surgery, for instance to evaluate the effectiveness of coronary bypass grafts.

Two experimental projects deal with basic mechanisms of epileptiform activity. The purpose of one is to determine whether GABA receptor antagonism is involved in the development of the acute epileptogenic effects of penicillin in the cerebral cortex of cat. The experimental design includes the use of multiple barrelled micropipettes for the extracellular recording from a single cortical neuron and simultaneous iontophoresis of penicillin, GABA, and other various amino acids of known excitatory and inhibitory action. These experiments, which are still in course, would seem to suggest that GABA receptor antagonism is unlikely to be the crucial mechanisms whereby penicillin exercises its epileptogenic effect. The other project attempts to investigate the role of deafferentation in the development of epileptic

activity. The experimental model utilizes the spinal trigeminal nucleus of cat several weeks following retrogasserian trigeminal rhizotomy and includes the study of unit firing patterns in both intact and deafferented nuclei, in resting conditions, following sensory stimulation and in response to various iontophoretically applied putative neurotransmitter agents. This series of experiments is still in course and the findings are too preliminary to permit any definite conclusion. Both these projects are to be continued through, and are expected to be completed within, the next fiscal period.

Of the two last projects carried out in collaboration with NS-LNNS, one deals with axonal injury and retrograde cell body reaction; the other with the effects of triiodothyronine on axonal regeneration. The two investigations are performed using both immature and mature rats. Both projects have been recently initiated and the specific objectives, methods and findings derived from preliminary observations are outlined in the related Project description.

Other Activities

The chief of the Section on Functional Neurosurgery is about to complete his Sabbatical leave at the Institute for Brain Research, University of Zurich. He has been actively involved in research projects dealing with the identification of electro-motor neurons in the spinal cord of fish (*Sternarchus albifrons*), and with the use of horse-radish peroxidase for identification of retrograde axonic flow. He is also preparing, in collaboration with Prof. Akert, an Atlas on "Membrane Morphology of the Central Nervous System of Vertebrates" which should appear in press at the end of 1976. The Chief of the Branch is also officially and actively involved in editorial duties of several specialized Journals (*Electroenceph. clin. Neurophysiol.*; *Epilepsia*; *Arch. Ital. Sci. Biol.*) and is co-editor of the Handbook of EEG and Clinical Neurophysiology which consists of about 30 volumes. As Past President and current member of the Executive Committee of the International Federation of Societies for Electroencephalography and Clinical Neurophysiology, he is actively involved in the organization and scientific program of the IX International Congress of said Federation to be held in Amsterdam (The Netherlands) in 1977.

ANNUAL REPORT

July 1, 1975 through June 30, 1976
Clinical Neurosciences Branch
National Institute of Neurological and Communicative
Disorders and Stroke

Cosimo Ajmone Marsan, M.D., Chief

Summary of Program Activity

This is the first Annual Report of this Branch which has been created recently as an amalgamation of the pre-existing Branch of Electroencephalography and Clinical Neurophysiology with part of the old Neurosurgical Branch. The new Branch consists of two sections: Functional Neurosurgery and Clinical Neurophysiology, the activities of the latter including, as in the past, a clinical-diagnostic service for all patients in the Clinical Center. In the overall evaluation of the activities of the Branch, and in particular of those of the Section on Functional Neurosurgery, it should be pointed out that these have been hindered by the absence of this section's chief (Sabbatical leave) as well as by the consequent unavailability of new clinical material through the entire fiscal period.

1. Clinical-Diagnostic Service

The activity related to this Service in the just completed fiscal period has involved a total of 3.8 man/years (0.8 professional and 3.0 technical-secretarial). This total, which is similar to that of the preceding fiscal period, is down from the over 5 man/years of recent years. The difference is due to the reduction in technical staff (2 EEG technologists versus 3-4 in the past). Whereas this reduction has not significantly affected the total number of EEG examinations, it has seriously interfered with the handling of emergency referrals, frequently requiring the cancellation or rescheduling of examinations (of particular inconvenience in the case of out patients) and limiting the service to the week-day's working hours. The occasional, unavoidable absence of one or both of the two technicians has, of course, accentuated these problems.

From the time the last Report was prepared (April 10, 1975) to that of the present Report (April 12, 1976) a total of 1310 EEG tracings have been obtained and interpreted, in patients referred to our laboratory as part of their routine clinical work-up or for specific research projects originating outside of this Branch and/or Institute. The distribution of these referrals according to the various Institutes of origin has been:

<u>INSTITUTE</u>	<u>NO.</u>	<u>%</u>
NINCDS (OP 358)	864	65.7
NIMH	196	15.0
NCI	107	8.2
NIAMD	62	4.8
NHLI	42	3.2
NIAID	28	2.1
NCHD	2	0.2
Misc.	9	0.8
TOTAL	1310	100.0

A considerable number of the 446 examinations requested by Institutes other than NINCDS had to be carried out on the ward, at the patient's bed or in the Intensive Care Unit. In addition to the EEG examinations, which include those performed in four patients with chronically implanted depth electrodes, three records were obtained directly from the exposed cortex in the course of surgical intervention. This unusually small number of non-routine procedures reflects the situation referred to in the introduction.

2. Research Activity

The present Report includes a total of 20 projects, 9 new and 11 representing a continuation of old projects. Of these, 7 are of a primary clinical nature while the other 13 are experimental. Two additional projects in which our professional staff have been actively involved are described in the Report of the Laboratory of Neural Control. Of the 20 projects, six have been completed while the others are expected to continue through or be completed within the coming fiscal period. A total of 14, related papers have been published or are currently in press.

a) Clinical

Work in the Functional Neurosurgery program has continued to provide new information of a fundamental nature regarding the human nervous system. Quantitative determinations of the density of Purkinje cells in the cerebellum of 7 epileptic patients were compared with those in 5 patients without epilepsy or neurologic disease. The controls showed a mean density of 3.56 Purkinje cells/linear mm. of cortex/10 μ section (SD = 0.18) while the epileptics had a mean density of 1.35 (SD = 0.82) cells. In all cases the loss of cells was accompanied by isomorphic gliosis. Three of the epileptics had devices for cerebellar stimulation implanted. Of these the patient showing the lowest count of Purkinje cells appeared to have the best degree of seizure control associated with stimulation. This curious situation raises question as to the role of Purkinje discharge in seizure control.

In fact, in another related study, carried out in epileptic patients receiving chronic cerebellar stimulation, it was shown that such a procedure (either unilateral alternating or bilateral) induces significant elevations in the levels of norepinephrine in their cerebrospinal fluid. These alterations in norepinephrine metabolism may be related to the seizure-suppression effect of cerebellar stimulation. In the meantime, a continuous follow-up study of the same patients with an effective method of double-blind stimulation would seem to indicate that in most subjects their seizure frequency tends to increase during periods of ineffective cerebellar stimulation. In spite of the fact that there has been no complete abolition of seizures, chronic stimulation appears to have unquestionable beneficial effects: the dosages of medical treatment has been reduced and the families of all patients are enthusiastic about the results since the patients have less frequent seizures and are more manageable and alert.

As part of the same project, two additional studies deal with the diagnosis and treatment of complex cases of medically intractable seizure disorders. Specifically these studies emphasize the use of chronically implanted recording electrodes and the information they provide for the localization of the epileptogenic process in patients with complex partial seizures and multiple or bitemporal "foci" and in patients with partial seizure originating in extratemporal structures. These and the preceding studies have been completed and related papers are either in press or have been published.

Another research project investigates the clinical ictal patterns in epileptic patients with electrographic evidence of epileptiform activity within the temporal areas. These seizures are rather complex and quite variable; the automatisms and vegetative symptoms predominate but motor patterns of both tonic and clonic nature are also observed. The availability of a relatively large number of carefully selected patients with a long follow-up and repeated EEG examinations offers an unparalleled opportunity to evaluate all their possible ictal patterns in detail. The findings, beside increasing our knowledge on the pathophysiology of "temporal lobe" seizures, complement those obtained in other groups of epileptics in the recent past and should prove of practical usefulness in the localizing diagnosis and classification of seizure disorders.

The opportunity offered by cortical exposure in the course of surgery for treatment of epilepsy has been utilized to study differences in oxidative responses to electrical stimuli in presumably pathological human cortex. This investigation based on the use of television fluorometry to monitor NADh changes, is expected to continue. Preliminary results and technical details have been published or are being prepared for publication. A summary is available in the description of the related project (01939b-05).

Another project deals with dyskinetic patients. In 5 of such cases undergoing thalamotomy through a frontal approach control radioenzymatic assays of lumbar CSF norepinephrine concentrations before and 12 days after surgery showed no significant alterations. Stimulation within the caudate nucleus, however, was followed by a significant fall in CSF norepinephrine concentration. This suggests that in man there are efferent noradrenergic pathways which are inhibited by stimulation in the caudate nucleus.

Clinical investigations were designed to identify brain mechanisms in man which mediate speech, language and memory. Gentle stimulation of the surface and depths of the human brain was used to plot distinct areas supporting linguistic behavior. In the posterior region, the vital language zone codes man's experiences and provides a mechanism for retrieving memory data. More anterior areas along the temporal lobe are not important in language and, instead, advance memory and learning by storing information. In the frontal region, there lies an area which is vital to the function of forming and uttering speech and articulation. In addition, this brain region regulates the rules of grammar and syntax in speech.

The subcortical relays to these areas, specifically the pulvinar nuclei, play a lesser role in language and memory. These nuclei enter stimulus processing at an early, pre-perceptual phase. Other subcortical systems like the cingulum do not influence cognitive processes directly, and seem to participate in directing behavior via emotional impetus to reinforce learning. In the right brain, these structures subserve different functions. In contrast to the verbal skills of the left hemisphere, the right hemisphere and subcortex employ visual, spatial strategies.

Brain-behavior relations in personality development and character formation were examined in terms of changes produced by different brain lesions. In a study of epileptic patients, individuals with left temporal insult displayed a poor self-image, tended to exaggerate their problems and to adopt a reflective, ideative posture. The right temporal epileptic patients were more self-assured, tended to deny personal problems and were described as very emotive.

In contrast to these studies where brain lesions were associated with intellectual disorders, "functional insult" via sensory deprivation had a disabling effect on neural development. Preliminary study of deaf individuals by non-invasive techniques revealed that the language brain under these conditions is less efficient in processing verbal information. These data argue for the reliance of language on auditory signals and the importance of cross-modal integration in language formation.

b) Experimental Research

A number of investigations deal with cortical metabolic changes in various experimental situations. Specifically they deal with the oxygen consumption, monitoring of blood flow, Hgb saturation and NADH fluorescence changes as well as with the release of potassium and its clearance in the cortex of different animals during and following local cortical stimulation, stimulation of the brain stem reticular formation, in the course of Metrazol-induced seizures and after the experimental production of chronic epileptogenic foci, as well as under normal or hypotensive conditions or during cerebral ischemia of various degrees.

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PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Epileptogenic Mechanisms in the Brain of Man and Other Primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: J.M. Van Buren, M.D.	Associate Chief	CN NINCDS
C. Ajmone Marsan, M.D.	Chief	CN NINCDS
OTHER: J.H. Wood, M.D.	Clinical Associate	SN NINCDS
B. Ludwig, M.D.	Clinical Associate	CN NINCDS
N. Mutsuga, M.D.	Visiting Fellow	CN NINCDS
F.T. Hambrecht, M.D.	Medical Officer	FNP NINCDS
M.G. Ziegler, M.D.	Pharmacologist	LCS NIMH
C.R. Lake, M.D.	Clinical Associate	LCS NIMH
J. Sode, M.D.	Endocrinologist	NNMC

COOPERATING UNITS (if any)

Laboratory of Neural Control, NINCDS
Laboratory of Clinical Science, NIMH
Medical Neurology Branch, NINCDS
~~Division of Endocrinology, National Naval Medical Center~~
LAB BRANCH

Clinical Neurosciences

SECTION

Functional Neurosurgery Clinical Neurophysiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

2

PROFESSIONAL:

1.8

OTHER:

0.2

SUMMARY OF WORK (200 words or less - underline keywords)

Quantitative studies of Purkinje cell population over Crus I & II near the midline showed significant atrophy of 3 individuals undergoing implantation of cerebellar stimulators & postmortem material from 4 other epileptics. Five normal autopsy specimens showed Purkinje populations nearly twice the epileptic. This finding brings into question the therapeutic rationale of cerebellar stimulation which is usually considered to be to increase the inhibitory discharge of the Purkinje cells.

Using a radioenzymatic assay method the norepinephrine content of the CSF was found to decrease following stimulation of the striatum in 5 patients undergoing stereotaxic thalamotomy. Control studies ruled out effects of simple surgical intervention. This suggested stimulation induced inhibition of noradrenergic pathways.

In patients with temporal lobe seizures subject to additional foci. In this complicated group, most cases would not have been accepted for temporal lobectomy without the use of depth electrodes. The additional information permitted the cure or significant improvement in some 25-50% of the patients with a follow-up averaging 8.2 years.

Project Description:**Objectives:**

1. To study causal mechanisms of epileptic seizures in man and other primates.
2. To study the electrographic characteristics of epileptogenic activity in the brain of man and other primates.
3. To study the approved methods of surgical therapy for these lesions and develop new therapeutic methods.
4. To make use of opportunities in diagnosis and therapy for the study of neurophysiological and neuropsychological problems.

Methods Employed:

1. Clinical neurological examination.
2. Special radiographic and other contrast examinations.
3. Electrographic, including electrocorticographic and stereo EEG examination.
4. Physiological and psychological techniques as indicated.
5. Histological and chemical examinations as required.

Major Findings:

a) A pilot study of the effects of chronic intermittent cerebellar stimulation was made on five individuals in whom the devices were implanted between March-July 1974. The only complications have been the chronic accumulation of CSF about the chest receivers due to difficulty in achieving a watertight seal about the wires as they pass through the dura of the posterior fossa. Two patients have required wound revisions to correct this, and one has had two wound revisions, yet with further recurrence. Intermittent elevations in blood pressure to borderline hypertensive levels have been noted since the onset of stimulation in all five patients. However, no cause and effect phenomena could be shown by blood pressure monitoring during periods of non-stimulation and stimulation.

All patients had previously had an average of 1+ fit/day and have been maintained on full medication. The Laboratory of Neural Control has provided support for testing the stimulation devices and calibrating the current output in situ during the

implantation operation. It has also provided a simple and effective method of double-blind stimulation. Preliminary results of seizure charting during the double-blind study suggest that four of the five patients have increases in seizure frequency during periods of ineffective cerebellar stimulation.

No patient has had complete suppression of all his seizures by chronic cerebellar stimulation however all have experienced partial seizure inhibition that appears additive to that caused by his anticonvulsant medications. Phenobarbital dosages have been reduced in all patients as compared with dosages taken prior to the surgical implantation of the cerebellar electrode. The families of all the patients have been enthusiastic about the results of stimulation on the basis that the patients have less seizures, are more alert, responsive etc. These improvements in mentation have been documented by psychometric testing and may be due to reductions in phenobarbital dosages.

b) Study of the cerebellum of epileptics

Quantitative studies of the Purkinje cell population in three of the patients with cerebellar stimulators show severe atrophy, with counts of 0.6, 1.0 and 2.6 Purkinje cells/mm. of cortical surface (Purkinje cells showing a nucleolus with counts adjusted for 10μ sections) with biopsies taken over the horizontal fissure in crus I and II about 1 cm. from the midline. Similar samples from four other epileptic brains in our collection showed counts of 0.7, 1.1, 1.3 and 1.6. Five normals varied from 3.3 - 3.8 cells/mm. surface.

If the rationale of cerebellar stimulation is to increase the inhibitory discharge of the Purkinje cells, this finding brings the basic rationale into question.

c) Study of cerebrospinal fluid neurochemical alterations induced by cerebellar stimulation in epileptic patients.

Lumbar cerebrospinal fluid norepinephrine concentrations were determined by radioenzymatic assay in four epileptic patients receiving chronic unilateral, alternating cerebellar stimulation. Stimulation was discontinued for seven days after which cerebrospinal fluid norepinephrine levels decreased significantly. Cerebrospinal fluid norepinephrine concentrations rose significantly after 16 hours of bilateral, continuous stimulation as compared to levels determined after the week without stimulation. Cerebrospinal fluid adenosine monophosphate levels determined by radioimmunoassay were not significantly altered by either mode of stimulation.

This neurochemical study suggests that both unilateral alternating and bilateral, continuous cerebellar stimulation induces significant elevations in steady-state cerebrospinal fluid norepinephrine levels. These alterations in norepinephrine metabolism may suppress seizures.

d) The air supported microelectrode developed by project No. Z01 NS 01687-06 LNLC has been subject to mechanical problems in application to the human brain during craniotomy for epilepsy. When it functioned reliably, the extracellular records were remarkable in permitting the continued recording from the same neuron (or small group) for many minutes despite the wide respiratory and cardiac pulsations of the cortex. A systematic study of the ECoG spike-extracellular unit activity interrelationship has as yet not been practical. The progress in design, however, is very encouraging.

e) Temporal-lobe seizures with additional foci treated by resection.

Epileptic patients with psychomotor seizures and epileptiform electrographic activity localized to one temporal region but with additional complicating foci (either suprasylvian or one the opposite side) have been studied with repeated scalp EEG examinations and, when possible, with chronically implanted electrodes. Temporal lobectomy was eventually carried out in such cases and it was found that this procedure renders a fair number of patients (between 25% and 50%) either seizure-free or with significant and useful reduction in their seizure frequency. The cure and improvement rates of cases followed up after temporal resection with or without prior study with implanted electrodes were approximately equal. However, the implanted electrodes permitted surgical treatment of certain cases which would have been rejected on the basis of evidence derived from the scalp recordings alone.

Of 28 of these 34 patients with persisting EEG epileptiform activity in the postoperative period, only one had such activity in a different location in a follow-up period of 6 years. No evidence of spreading epileptic activity or appearance of "mirror foci" was seen during a follow-up period averaging 8.2 years. Seizure remission up to 15 years with eventual recurrence of the original seizure type may occur following surgical therapy. Follow-up studies of surgical epileptic treatment of less than 3 to 5 years are of doubtful value.

f) Depth and direct cortical recording in seizure disorders of extratemporal origin.

This study is based on 28 patients with intractable seizures in whom exclusively extratemporal or a combination of temporal and

extratemporal electrodes were chronically implanted for the localization of their epileptogenic process and possible surgical treatment. Mainly on the basis of data derived from this particular technique of investigation, surgical treatment was eventually carried out in 14 patients. It is concluded that the use of implanted electrodes in seizure disorders of probable extratemporal origin can be of real diagnostic benefit in certain specific situations. In most instances, however, this technique simply serves to demonstrate the complexity of an apparently simple case or, of greater clinical consequence, might tend to over simplify cases which are actually very complex. Indeed, many data in this study raise some doubts about the validity of the classical concepts of "focal" epilepsy.

g) Functional localization is supplementary motor area.

In 9 patients with seizures it was possible to restudy the supplementary motor area with implanted depth electrodes controlled radiologically. Although the results confirmed the representation of gross motor movements of a postural adjustment type in this region, the contraversive head turning, long considered a part of this movement complex did not appear from stimulation of the medial hemisphere although often obtained from stimulation of descending fibers from the premotor region of the lateral hemisphere. In addition sensory responses were often obtained. These were frequently of an ipsilateral or bilateral referral and involved large areas of body usually with a proximal distribution. These new findings may assist in interpreting the localizing significance of observed clinical seizures.

Publications:

Wood, J.H., Ziegler, M.G., Lake, R.C., Sode, J., Brooks, B.R., Van Buren, J.M.: Elevations in cerebrospinal fluid nor-epinephrine during unilateral, alternating and bilateral, continuous cerebellar stimulation in man. J. Neurosurg.: in press, 1976.

Rajjoub, R.K., Wood, J.H., Van Buren, J.M.: Significance of Purkinje cell density in seizure suppression by chronic cerebellar stimulation. Neurology (Minneap.): In press, 1976.

Van Buren, J.M., Ajmone Marsan, C., Mutsuga, N.: Temporal-lobe seizures with additional foci treated by resection. J. Neurosurg. 43: 596-607, 1975.

Ludwig, B.I., Ajmone Marsan, C., Van Buren, J.M.: Depth and direct cortical recording in seizure disorders of extra-temporal origin. Neurology: in press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 00200-22 CN
PERIOD COVERED: July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) Involuntary Movements		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: J. M. Van Buren, M.D. P. Fedio, Ph.D. J. Wood, M.D. OTHER: C.R. Lake, M.D. M.G. Ziegler, M.D. I. Shoulson, M.D. B.R. Brooks, M.D.	Associate Chief Research Psychologist Clinical Associate Clinical Associate Pharmacologist Clinical Associate Clinical Associate	CN NINCDS CN NINCDS SN NINCDS LCS NIMH LCS NIMH IR NINCDS MNB NINCDS
COOPERATING UNITS (if any) Laboratory of Clinical Science, NIMH Medical Neurology Branch, NINCDS		
LAB/BRANCH Clinical Neurosciences		
SECTION Functional Neurosurgery		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.7	OTHER: 0.2
SUMMARY OF WORK (200 words or less - underline keywords) Cerebrospinal fluid <u>norepinephrine</u> concentrations were preoperatively determined in five patients. Stereotaxic <u>thalamotomy</u> was performed using depth coagulating electrodes. No significant alterations in pre-stimulation lumbar cerebrospinal fluid norepinephrine levels were noted twelve days after electrode installation. Twelve hours after intermittent electrical <u>stimulation</u> of the <u>caudate nucleus</u> , lumbar cerebrospinal norepinephrine concentration was significantly decreased suggesting stimulation-induced inhibition of noradrenergic pathways. In nine <u>Huntingtonian</u> patients with caudate atrophy norepinephrine levels were significantly lower than those noted in nine age-and sex-matched control patients.		

Project Description:

Objectives: Neurological disease characterized by dyskinesias offers a two-fold opportunity for research. The pathological aspects of the disease itself may be studied as well as the pathophysiology of the motor system. The second aspect is the unparalleled opportunities afforded for neurophysiological and biochemical studies in man by stereotaxic surgery in the treatment of dyskinesias. Studies made directly of the disease itself have not received great emphasis in the present research since they require biochemical and pathological support.

Methods Employed: Depth electrode stimulation employs specially built electrodes and a current-monitored stimulator. Norepinephrine content in cerebrospinal fluid is determined by radioenzymatic assay techniques.

Major Findings: Lumbar cerebrospinal fluid norepinephrine concentrations were preoperatively determined in five patients using a radioenzymatic assay technique. Stereotaxic thalamotomy was performed using depth coagulating electrodes with stimulating points at 5.0 mm intervals along the shaft. No significant alterations in prestimulation lumbar cerebrospinal fluid norepinephrine levels were noted twelve days after electrode installation. Stimulating points within the caudate nucleus were anatomically localized using ventricular landmarks and stimulation-induced neurophysiological response were recorded. Twelve hours after intermittent electrical stimulation of the caudate nucleus, lumbar cerebrospinal norepinephrine concentration was significantly decreased suggesting stimulation-induced inhibition of noradrenergic pathways.

Lumbar cerebrospinal fluid norepinephrine concentrations were determined by radioenzymatic assay in nine Huntingtonian patients with caudate atrophy. These norepinephrine levels were significantly lower than those noted in nine age- and sex-matched control patients.

Significance to Bio-Medical Research and the Program of the Institute: The use of a human subject who is cooperative and unsedated permits many tests of sensory and psychological function which are impossible in lower animals, even with time-consuming conditioning experiments. The use of the human subject is, therefore, not a duplication of animal experimentation but an extension of this.

Proposed Course of the Project: Dyskinesia patients will be studied as they become available in support of projects related to neuropsychological biochemical studies. At the present time the personnel support does not permit further studies utilizing the neurophysiological opportunities offered by the surgical approach.

Publications:

Wood, J.H., Lake, C.R., Ziegler, M.D.: Neurophysiologic and neurochemical alterations during electrical stimulation of human caudate nucleus. J. Neurosurg.: In press, 1976.

Wood, J.H., Ziegler, M.G., Lake, C.R., Shoulson, I., Brooks, B.R.,
Van Buren, J.M.: Cerebrospinal fluid norepinephrine reductions in man after
degeneration and electrical stimulation of the caudate nucleus. Arch. Neurol.:
in press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 00304-21 CN
PERIOD COVERED: July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) Effect of Lesions upon the Function and Structure of the Human Central Nervous System		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: J.M. Van Buren, M.D. Associate Chief CN NINCDS		
OTHER: R.C. Borke Biologist CN NINCDS P. Fedio, Ph.D. Research Psychologist CN NINCDS		
COOPERATING UNITS (if any) none		
LAB/BRANCH Clinical Neurosciences		
SECTION Functional Neurosurgery		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 2.4	PROFESSIONAL: 0.3	OTHER: 2.1
SUMMARY OF WORK (200 words or less - underline keywords) <p> Further studies of the <u>thalamic afferents</u> fiber from the <u>posterior column</u> and <u>superior cerebellar peduncle</u> show in chimpanzee that they initially enter the posterior and anterior portions of the <u>n. ventralis posterior (VP)</u> respectively rather than the VP and <u>n. ventralis lateralis</u>. In a human case, a discrete thalamotomy on <u>one side</u> entered the posterior part of VP (<u>n. ventrocaudalis</u>) producing <u>contralateral paresthesias</u>. A lesion in the anterior part of VP (<u>n. ventrointermedius</u>) resulted in no subjective sensory change supporting the non-sensory nature of this portion of VP in man. </p> <p> <u>Stimulation</u> of the <u>supplementary motor area</u> in man produced the expected contraversive gross muscular synergies, but paramedian stimulation failed to elicit the classical contraversive movements of the head. Frequent sensory responses were found most frequently involving large areas or ipsilateral regions of the body. These new findings may assist the interpretation of clinical seizure phenomena. </p>		

Project Description:

Objectives: This project is directed toward the study of basic neuroanatomy and neurophysiology in man, making use of pathological material and the opportunities for study afforded by the operative treatment of neurological disease.

Methods Employed:

Anatomical studies:

1. Serial sections of human and animal brains in celloidin for myeline and Nissl series.
2. Section and staining of primate brains with Nauta technique for demonstration of degenerating pathways.

Major Findings: Due to the absence of the principal investigator the patient phase of this project has been temporarily postponed.

Lesions were placed in the pre and post central gyri and the parietal lobe to the P-0 fissure in six chimpanzee preparations. Frozen sections of Nauta and Fink Heimer techniques have been prepared. Analysis of the terminal anterograde degeneration in the thalamus is being completed. These cortico-thalamic projections will be correlated in the ascending brain stem and cerebellar projections to the lateroventral regions of the thalamus.

Proposed Course of Project: The availability of new techniques for the study of enzyme histochemistry offers the exciting prospect of having the means to undertake topographical studies in high primates of metabolic processes and relate the relative activities to the cytoarchitectural entities of the thalamus, basal ganglia and raming diencephalon and brain stem. Once these basic maps are made up the technique can be applied to other studies such as that of the experimental epilepsy (see Project No. Z01 NS 00100-23 CN).

The problems, of course, center upon the specialized talent as well as time and space required to produce the "stains." Since we cannot hope to obtain this talent within the Branch, it is obvious that some type of collaborative work must be undertaken. We are fortunate in having access to Dr. Bloom at St. Elizabeth's Hospital and Dr. Kaufmann in Building 35 (who has a preparation to detect tyrosine hydroxylase). Particular interest has been paid to Dr. Roberts who can selectively label glutamic acid decarboxylase with peroxidase, which would apply well to the

epileptic project. The logistics of collaboration here are difficult since he resides in California. The possible use of a tritium label opens further possibilities for topographical studies. With the exception of one paper from Dr. Roberts' group, no studies of this type were reported at the meeting of the American Association of Anatomists, indicating our opportunity to advance rapidly in this field if proper liaison can be made. Personnel training in electron microscopy is continuing.

Publications:

Van Buren, J.M., Borke, R.C., Modesti, L.M.: The sensory and nonsensory portions of the nucleus "Ventralis Posterior" thalami of chimpanzee and man. J. Neurosurg. in press, 1976.

Van Buren, J.M. and Fedio, P.: Functional representation on the medial aspect of the frontal lobe in man. J. Neurosurg. 44: 275-289, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01245-11-CN
PERIOD COVERED: July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) EEG Learning Correlates Using Scalp and Intracranial Depth Electrodes		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	P. Fedio W. Sheriff M. Buchsbaum J. Van Buren A. K. Ommaya	Research Psychologist Computer Programmer Research Medical Officer Associate Chief Acting Chief CN NINCDS TD NINCDS AP NIMH CN NINCDS SN NINCDS
COOPERATING UNITS (if any) Section on Technical Development, NIMH; Adult Psychiatry Branch, NIMH		
LAB/BRANCH Clinical Neurosciences		
SECTION Functional Neurosurgery		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2
SUMMARY OF WORK (200 words or less - underline keywords) <u>Central, neural processing</u> of information by the human brain was monitored by <u>averaged evoked response</u> techniques. The electrographic recording of left and right brain activity during <u>learning</u> and <u>perception</u> in normal subjects was compared with that of neurosurgical patients. Suspect disturbances in <u>brain-behavior</u> relations in psychiatric patients were evaluated, relating <u>left brain</u> dysfunction to ideational disorders, <u>right brain</u> , to <u>emotional problems</u> .		

Project Description:

Objectives: To identify brain mechanisms in man which subserve perception, and the storage and retrieval of information; to evaluate the significance of brain dysfunction in psychiatric patients.

Methods Employed: A series of verbal and nonverbal tasks, designed to evaluate left or right brain processes, were used. Electroencephalographic (EEG) activity was recorded from scalp electrodes positioned over the posterior temporal-parietal regions of the left and right hemisphere. Included for study were neurosurgical patients who had undergone unilateral removals of the temporal lobe, and psychiatric patients exhibiting affective or ideational thought disorders.

Major Findings: All electrographic test runs were conducted off-line and the evoked potential data for cognitive parameters is currently being processed. The study involving neuropsychiatric patients is in progress.

Significance to Biomedical Research and the Program of the Institute: Behavioral data available from epileptic patients following unilateral temporal lobectomy reveal significant perceptual and learning deficits which are related to the laterality of surgery and to the specific character of the material. The technique employed in this project affords a more precise method for outlining cortical and subcortical systems in the human brain which mediate learning and memory. The research also provides physiologic and behavioral data for the comparison of neurologic and psychiatric patients in order to identify possible brain dysfunctioning in schizophrenia or psychosis.

Proposed Course of the Project: To develop additional neuropsychological tasks and biometric programs for EEG analysis with wider application to patients with different neurologic or psychiatric disorders.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01424-10-CN

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Response Modulation by the Limbic System in Man: Neuropsychological and Physiological Changes.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. M. Bear	Surgeon, PHS	CN NINCDS
	P. Fedio	Research Psychologist	CN NINCDS
OTHER:	J. Van Buren	Associate Chief	CN NINCDS
	A. Ommaya	Acting Chief	SN NINCDS

COOPERATING UNITS (if any)

Division of Biometry, NIMH; DCRT; Medical Neurology Branch, NINCDS; LaFayette Clinic, Detroit, Mich.; Livingston Epilepsy Clinic, Baltimore, Md.

LAB/BRANCH

Clinical Neurosciences

SECTION

Functional Neurosurgery

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

1.7

PROFESSIONAL:

1.2

OTHER:

0.5

SUMMARY OF WORK (200 words or less - underline keywords)

Behavioral characteristics are studied in patients with temporal lobe epileptic foci. Patients and raters independently complete true-false questionnaires which probe specific features of behavior and emotion, and permit analysis of distortions in self perception. Temporal epileptic patients are compared with matched normal subjects and patients with other neurologic illnesses. Patients with a right temporal focus are contrasted with left temporal epileptics. Statistical analyses are employed to codify behavioral profiles of right and left temporal epileptic subjects. The research examines the role of the anterior temporal lobe in establishing limbic associations and differences between the left and right hemispheres in regulating emotions in man.

Project Description:

Objectives:

1. To determine whether an epileptic focus in the human temporal lobe produces specific behavioral effects.
2. To construct a behavioral profile which effectively differentiates temporal epileptic patients from others.
3. To compare self perceptions of behavior and emotions with evaluations made by observers for each of the sampled groups.
4. To contrast the behavioral profiles of patients with right vs. left temporal lobe focus.

Methods Employed: From a review of the literature and preliminary testing, 18 behavioral traits said to appear commonly among temporal lobe epileptics were assessed by 2 true-false questionnaires: a Personal Inventory completed by each subject (Self-Report) and a Personal Behavior Survey completed about each subject by a longtime observer (Rater-Report).

Four groups of subjects have been tested: (1) right temporal epileptics (N=15), (2) left temporal epileptics (N=12), (3) patients with progressive neuromuscular disorders (N=9), (4) normal subjects (N=12).

Major Findings:

1. The epileptic patients were distinct both in overall scores and trait profiles from either normal or other neurologic subjects. These group differences were independently significant in self reports and rater evaluations. Although there was no consistent relationship between seizure frequency and behavior, a long duration of seizure disorder was associated with the most aberrant profiles.
2. Multivariate stepwise discriminant analysis identified specific traits which differentiated epileptics from nonepileptics. Based on patient self reports, the traits of humorless sobriety, dependence, and obsessionalism constituted a discriminant profile of the temporal epileptic subjects. From the raters' evaluations, circumstantiality, philosophical preoccupation, and anger were seen as distinctive features. The majority of traits evaluated (14/18) were characteristic of the temporal epileptic subjects.
3. Rater observations yielded markedly different profiles for the left and right temporal patients. The right temporal patients exhibited emotive traits: anger, sadness, emotionality, obsessionalism, circumstantiality. The left temporal subjects were characterized by ideational tendencies: nascent philosophical interests, sense of personal destiny, religiosity.

4. Only the temporal epileptic groups demonstrated significant distortions of self-perception. Right temporal patients specifically denied "socially undesirable behavior. Left temporal subjects presented a contrasting "catastrophic" exaggeration of socially disapproved tendencies while minimizing desirable, conscientious behavior. In general, right temporal patients enhanced their self images, while left subjects tended to view themselves negatively.

Significance to Biomedical Research and the Program of the Institute.

By identifying specific behavioral sequelae of a temporal lobe focus, these observations further neuroanatomical understanding of emotional processes. The results may be interpreted as a consequence of enhanced sensory-limbic associations. This interpretation regarding the effects of temporal lobe epilepsy in human subjects is consistent with extensive animal experimentation on sensory-limbic disconnections. The findings quantitatively support an asymmetry of emotional processing within the right and left hemispheres of man.

Proposed Course of Project: Testing of additional psychiatric and neurologic contrast groups (nontemporal epileptics) is planned.

Publications:

Bear, D.: Temporal Lobe Epilepsy - A Syndrome of Sensory Limbic Hyper-connection; Cortex (In Press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01658-09 CN																
PERIOD COVERED July 1, 1975 to June 30, 1976																		
TITLE OF PROJECT (80 characters or less) Hemispheric Development and Specialization of Intellectual Functions																		
NAMES, LABORATORY AND INSTITUT AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																		
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">P. Fedio</td> <td style="width: 35%;">Research Psychologist</td> <td style="width: 15%;">CN NINCDS</td> </tr> <tr> <td></td> <td>J. Van Buren</td> <td>Associate Chief</td> <td>CN NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>G. R. Frederick</td> <td>Psychologist</td> <td>CN NINCDS</td> </tr> <tr> <td></td> <td>M. Mishkin</td> <td>Psychologist</td> <td>LP NIMH</td> </tr> </table>			PI:	P. Fedio	Research Psychologist	CN NINCDS		J. Van Buren	Associate Chief	CN NINCDS	OTHER:	G. R. Frederick	Psychologist	CN NINCDS		M. Mishkin	Psychologist	LP NIMH
PI:	P. Fedio	Research Psychologist	CN NINCDS															
	J. Van Buren	Associate Chief	CN NINCDS															
OTHER:	G. R. Frederick	Psychologist	CN NINCDS															
	M. Mishkin	Psychologist	LP NIMH															
COOPERATING UNITS (if any) Neurological Surgery, University of Washington, Seattle, Washington; National Technical School for the Deaf, Rochester, New York; Laboratory of Psychology, NIMH; Aphasia Research Unit, Boston VA Hospital, Boston, Massachusetts																		
LAB/BRANCH Clinical Neurosciences																		
SECTION Functional Neurosurgery																		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014																		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																
2.1	0.6	1.5																
SUMMARY OF WORK (200 words or less - underline keywords) The disabling effects of <u>brain damage</u> in man were evaluated on a broad range of <u>perceptual</u> , <u>learning</u> and <u>memory</u> functions. Changes in the intellectual behavior of neurologically handicapped individuals were evaluated before and after <u>brain surgery</u> and during <u>electrical stimulation</u> of the surface and depths of the brain. In contrast to these cases with confirmed brain injury, the effects of peripheral, <u>sensory deficits</u> were assessed in terms of possible neuropsychological dysfunctioning, and <u>communicative disorders</u> .																		

Project Description:

Objectives:

1. Outline brain mechanisms which support speech and language functions, and code information to be held for immediate (short-term) or for delayed (long-term) memory to assess the effects of injury to these brain regions on communication skills and memory.
2. Compare the effects of injury to the brain with conditions wherein there are sensory restrictions to language development as with congenital deafness.
3. Compare the effects of brain lesions during infancy vs. adulthood on the neuropsychological development and recovery of intellectual abilities.

Methods Employed:

1. The laterality and general outline of cortical zones instrumental in perception and immediate memory were mapped by stimulation of the cortex during neurosurgical treatment of epileptic patients. The behavioral tests utilized verbal or nonverbal materials: photographs of common objects or complex visual patterns. In each case, the subject was instructed to name the object, or to discriminate several patterns; a short-term memory command was also included for each task.
2. The organization of cortical and subcortical systems which enable man to speak and to use language or to remember events over varying periods of time was studied during electrical stimulation with therapeutic electrodes in the thalamus.
3. In collaboration with Dr. George Ojemann at the University of Washington, a study was designed to evaluate the role of cortical speech areas in grammatical or syntactical organization of language. Electrical stimulation was applied over the frontal (Broca) speech cortex during performance by patients on various tests of grammar.
4. The cerebral organization and lateral representation of language in the brain of deaf individuals will be evaluated by non-invasive, behavioral techniques. The testing procedure uses a tachistoscope, an apparatus which projects visual material at high speed (msec) onto the left or right visual fields, which in turn transmit the information directly to the left or right brain for processing. Accuracy of recognition for stimuli appearing in the left or right field reflects the efficiency of processing by left or right hemisphere.

Major Findings:

1. The ability to identify objects and to remember their names was studied during electrical stimulation of the exposed surface of the brain in patients undergoing surgery for the relief of epilepsy. This speech mapping procedure identifies by stimulation those cortical areas of the brain that are essential for the preservation of language. This approach prompted us to develop a nonverbal or right brain test paradigm involving discrimination and memory of complex visual patterns.

The general findings conform to established observations that language and related verbal processes rely upon an intact left brain. In the posterior temporo-parietal regions (Wernicke's area) of the left hemisphere, we were able to map a distinct zone which is indispensable for identifying verbal material. There was a disruption of language, the patient being unable to find the name of simple objects. Stimulation of the left frontal cortex (Broca's area) also interfered with object naming, albeit to a lesser degree. Stimulation of Broca's area did not prevent the patient from recognizing or remembering objects but from stating the name until after stimulation was terminated.

Whereas the classic deficit in language with Wernicke's aphasia emerges as an inability to name, this process is preserved when Broca's area is stimulated. Instead, speech production is upset. Broca's aphasia, however, may also include low level linguistic problems, such as disturbances in grammar. With Dr. Ojemann, we have shown that stimulation over Broca's area prevents patients from being able to formulate comparatives or to convert nouns into action verbs.

2. Stimulation of the right hemisphere, while sparing verbal processes, interfered with the patients' ability to match visual patterns. As with naming errors, the occurrence of visual perceptual errors was coincident with stimulation of the right posterior, but not the anterior temporal surface.

3. In each cerebral hemisphere, memory presses the anterior and posterior temporal regions into different support services. The primary or immediate memory system may be intimately linked with the anterior temporal lobe and medial structures (hippocampal complex), while the posterior temporo-parietal cortex may support secondary or long-term memory. Our findings correlate anterograde memory errors with stimulation of the anterior temporal lobe, and retrograde errors with posterior temporal stimulation. With anterior cortical stimulation, information could be retrieved from an immediate memory store, but newly perceived information could not be deposited into the same storage system for subsequent recall. This may be a defect in the consolidation rather than retrieval mechanism in that stimulation prevents the memory trace from being established for immediate recall.

In contrast, with cortical stimulation of the posterior temporal region, recently stored information could not be retrieved even though other

information could be simultaneously transferred into the same storage system. This indicates that, in man, memory for recent and remote information may be served by a common retrieval mechanism. Therefore, patients who suffer stroke or other forms of insult which invade the primary language zones are apt to display aphasia and a significant memory disorder.

3. Mechanisms for perception and memory were probed by electrical stimulation via therapeutic electrodes in the lateral thalamus. Stimulation within the left pulvinar nucleus induced transient dysphasia and a retrograde loss in recent memory for verbal memoranda. In contrast, comparable stimulation of the right pulvinar failed to disrupt verbal behavior and instead, interfered with perception and recognition of complex visual patterns. The findings suggest that an asymmetry in the functional organization of linguistic and nonverbal processes appears to exist at the level of the lateral thalamus.

At first glance, the left and right pulvinar nuclei behave like the ipsilateral cortex with regard to identifying, registering and retrieving from immediate memory, verbal and nonverbal material.

However, the behavioral deficits accompanying cortical and subcortical stimulation appear to be different. Specifically, the data suggest that dysphasia and amnesia were inseparable during cortical, but not thalamic stimulation. That is, if the patients were unable to name objects during cortical stimulation, they also experienced severe memory impairment. With thalamic stimulation, recent memory was occasionally spared while at the same time, the patient experienced dysphasia. This suggests that the registers or stores vital for coding information are dependent upon cortical mechanisms. The contribution of the pulvinar, and other posterior thalamic nuclei may be limited to pre-perceptual processing, that is, attenuating and shuttling sensory information to the cortical stations for interpretation and analysis.

4. The study of the operational efficiency of the 'language brain' in individuals who have been deprived of auditory experiences by congenital deafness, is in the preliminary phase. Individuals who became deaf before language skills were acquired (pre-lingual) are impaired neurolinguistically. Whether the loss is due to the absence of auditory input into the language brain or to the absence of cross modal integration, that is, auditory - visual, requires further investigation.

5. In collaboration with Dr. Butters, Aphasia Unit, Boston VA Hospital, patients with parietal lesions or Korsakoff's Syndrome (VA Hospital), or with temporal lobe lesions (NINCDS) are being studied on a wide range of memory tests. The initial data show that Korsakoff patients (with presumed subcortical or limbic damage) are more susceptible to the effects of distraction during learning than patients with cortical injury regardless of the type of material to be memorized. Experimental manipulation of learning trials (massed vs. spaced practice) also showed the Korsakoff patients to be more vulnerable to the effects of proactive inhibition.

Significance to Biomedical Research and the Program of the Institute:

The investigations contribute to the basic understanding of the development and organization of structural-functional relationships in the human central nervous system. This research advances clinical knowledge of the relationships between brain dysfunctions and amnesia, dysphasia, dyslexia and kindred communicative disorders.

Proposed Course of the Project: A battery of tests is being designed to examine adaptive strategies used by neurologic or sensory handicapped patients to compensate for visuomotor or language disorders. Visual and auditory tasks will be developed to further delineate immediate and long-term memory impairment in patients with lateralized cortical and subcortical lesions. Parallel studies of interhemispheric relations will be made during deep brain stimulation and during cortical stimulation of patients in the neurosurgical operating suite.

Publications:

Fedio, P.: The Cortical and Thalamic Mechanisms of Memory. Bulletin of the International Neuropsychology Society, January, 1976.

Rosenthal, L. S. and Fedio, P.: Recognition Thresholds in the Central and Lateral Visual Fields Following Temporal Lobectomy. Cortex 11: 217-229, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01939 -05 CN															
PERIOD COVERED July 1, 1975 through June 30, 1976																	
TITLE OF PROJECT (80 characters or less) NADH fluorometry in human cerebral cortex																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0"> <tr> <td>PI: D.V. Lewis, M.D.</td> <td>Clinical Associate</td> <td>CN NINETS</td> </tr> <tr> <td>OTHER: W.H. Schuette</td> <td>Biomedical Engineer</td> <td>DEL DSC</td> </tr> <tr> <td>W.C. Whitehouse</td> <td>Section Head</td> <td>PED CC</td> </tr> <tr> <td>C. Ajmone Marsan, M.D.</td> <td>Chief</td> <td>CN NINCDS</td> </tr> <tr> <td>J.M. Van Buren, M.D.</td> <td>Associate Chief</td> <td>CN NINCDS</td> </tr> </table>			PI: D.V. Lewis, M.D.	Clinical Associate	CN NINETS	OTHER: W.H. Schuette	Biomedical Engineer	DEL DSC	W.C. Whitehouse	Section Head	PED CC	C. Ajmone Marsan, M.D.	Chief	CN NINCDS	J.M. Van Buren, M.D.	Associate Chief	CN NINCDS
PI: D.V. Lewis, M.D.	Clinical Associate	CN NINETS															
OTHER: W.H. Schuette	Biomedical Engineer	DEL DSC															
W.C. Whitehouse	Section Head	PED CC															
C. Ajmone Marsan, M.D.	Chief	CN NINCDS															
J.M. Van Buren, M.D.	Associate Chief	CN NINCDS															
COOPERATING UNITS (if any) Biomedical Engineering Branch, Division of Research Services Television Engineering Section, Clinical Center																	
LAB/BRANCH Clinical Neurosciences																	
SECTION Functional Neurosurgery Clinical Neurophysiology																	
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014																	
TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.2	OTHER: 0.1															
SUMMARY OF WORK (200 words or less - underline keywords) In 8 patients undergoing excision of <u>epileptogenic lesions</u> , 70 observations were made. In 42 instances <u>NADH</u> levels changed 3-15% in response to <u>cortical stimulation</u> . Studies of spread of NADH change indicate that this does not extend outward symmetrically from the region of stimulation but may vary from area to area in an asymmetrical fashion. These variable responses may reflect local tissue changes related to the epileptogenic process.																	

Project Description:

Objectives: Use of the television fluorometry system previously developed to study differences in oxidative response to electrical stimuli in normal and abnormal human cortex.

Methods Employed: Using the previously described television system, fluorescence changes are recorded on video tape over a wide area of brain which has been exposed in the course of neuro-surgical procedures. Energy metabolism is evaluated through analysis of transients both spontaneously occurring and induced by electrical stimulation.

Major Findings: Of 17 areas surveyed in 8 patients undergoing craniotomy and excision of epileptogenic lesions, a total of 70 observations were made. In 42 instances NADH levels changed 3-15% in response to 5 second intervals of cortical stimulation. Although a rough correlation was seen between the quantity of current delivered (ma x sec) and the NADH change, this varied from case to case. The presence of cortical afterdischarge to stimulation often, but not invariably correlated with a greater percentage change in NADH levels.

In 4 patients showing short runs of low frequency (0.2/sec) spontaneous spikes, averaging of the NADH response for 5 sec following each of 5-30 spikes failed to demonstrate concomitant NADH changes. A similar lack of change was found in 4 patients in whom averaging was carried out with 22 to 40 low frequency penicillin spikes induced in cortex destined for excision.

Preliminary studies of the topography of spread of NADH change indicate that this does not extend outward symmetrically from the region of stimulation in human epileptogenic cortex but may vary from area to area in an asymmetrical fashion.

These variable responses may reflect local tissue changes related to the epileptogenic process (i.e. tissue unable to store NADH at normal levels due to structural change or drain by a local seizure process).

Proposed Course of the Project: Project provisionally completed. One paper published, another in preparation. Future of project uncertain depending on availability of equipment and staff.

Publications:

Schuette, W.H., Lewis, D.V., O'Connor, M. and Van Buren, J.: The design and operation of a dual-beam long-focal-length fluorometer for monitoring the oxidative metabolism in vivo. Med. & Biol. Engin. 1975, 14: 235-238.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02213-01 CN
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) Neuron response to axon injury in the immature and the mature rat		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Rosemary C. Borke Biologist CN NINCDS OTHER: M.W. Brightman, Ph.D. Section Head LNNS NINCDS		
COOPERATING UNITS (if any) Laboratory of Neuropathology and Neuroanatomical Sciences, NINCDS		
LAB/BRANCH Clinical Neurosciences SECTION Functional Neurosurgery		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER:
SUMMARY OF WORK (200 words or less - underline keywords) Ligation of the <u>hypoglossal nerve</u> at 7 days post partum results in irreversible <u>chromatolysis</u> and subsequent cell death of neurons of <u>hypoglossal nucleus</u> . In contrast, ligation of the hypoglossal nerve in 3 week old animal is accompanied by reversible chromatolysis with functional recovery of the affected neurons within 45-55 days following the <u>axonal injury</u> . The use of these two maturity states therefore will provide a model for further investigations of the basic sequence of how the cell body changes following injury to axon relate to the processes of <u>nerve regeneration</u> and <u>degeneration</u> .		

Project Description:

Objectives:

1. To compare the ultrastructural features of retrograde responses to nerve crush and ligation in a system capable of regeneration (three-week-old rat) with a system incapable of regeneration (one-week-old rat).
2. To study the sequency of events of the progressive change in the perikaryon capacity to respond to axonal injury.
3. To study the ultrastructural mechanism by which neurons are switched to a different metabolic program from the operating during the cortical maturation period of neurons.
4. To compare the glial reaction to axon injury in immature and mature animals.
5. To correlate the cell body responses with axonal regeneration in cells capable of regeneration (young adults) and cells incapable of regeneration (newborn - 7 days).
6. To study the membrane events of the axon and cell body of neurons subjected to nerve crush and ligation.

Methods Employed:

1. Surgical techniques of ligation and nerve crush of hypoglossal nerve in rats.
2. Transmission Electron Microscopy of the ultrastructural changes associated with axon regeneration and retrograde resection of the cell body.
3. Electron staining using tannic acid to delineate alterations in surface membrane coats of synapses on soma and dendrites of hypoglossal nucleus cells.
4. Freeze fracture techniques of hypoglossal nucleus and nerve in normal and injured neurons to study the membrane events associated with the retrograde responses.
5. Neurophysiological stimulation techniques as needed to test functional regeneration of axons.

Major Findings: This study was initiated recently, therefore significant findings are not available at this time.

Preliminary experiments have been conducted to determine a model in which the cells are capable of nerve regeneration and one in which the neurons are incapable of regeneration. The results of these experiments demonstrate that the CNS of neonatal rat is at a birth maturity spectrum between the immature state of hamster CNS and the mature state of rabbit CNS. Ligation of the hypoglossal nerve at 7-days post partum results in irreversible chromatolysis and subsequent cell death of neurons of hypoglossal nucleus. In contrast, ligation of the hypoglossal nerve in 3-week old animal is accompanied by reversible chromatolysis with functional recovery of the affected neurons within 45-55 days following the axonal injury. The use of these two maturity states therefore will provide a model for further investigations of the basic sequence of how the cell body changes following injury to axon relate to the processes of nerve regeneration and degeneration.

Proposed Course of the Project: To continue as described into the next fiscal year.

Publications: none.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02214-01 CN
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) Effects of 3, 5, 3'-Triiodo-L-Thyronine on cell body and axons following injury to hypoglossal nerve.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Rosemary C. Borke Biologist CN NINCDS OTHER: M.W. Brightman, Ph.D. Section Head LNNS NINCDS		
COOPERATING UNITS (if any) Laboratory of Neuropathology and Neuroanatomical Sciences, NINCDS		
LAB/BRANCH Clinical Neurosciences		
SECTION Functional Neurosurgery		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER:
SUMMARY OF WORK (200 words or less - underline keywords) Preliminary experiments are in progress to determine a drug dosage level which ensures maximum <u>protein synthesis</u> without toxicity to the animal over long term administration. The effects of <u>Triiodothyronine</u> (T3) will be studied in models capable of regeneration as well as those in which regeneration is limited. Studying the effects of T3 on these two different systems affords the opportunity to determine the basic mechanism of action of T3 on <u>axonal regeneration</u> .		

Project Description:

Objectives:

1. To study the effect of Triiodothyronine (T3) on the initiation and enhancement of the cell body response to axonal regeneration.
2. To determine if T3 (a hormone known to stimulate protein synthesis) may encourage regeneration in neurons whose regeneration capacity is limited.
3. To study the effects of T3 on events which are apart of the axon reaction of injured neuron (for example: glial reaction, retraction of dendrites).
4. To compare the membrane events associated with injury to hypoglossal nerve with those of nerve injury and administration of T3.
5. To determine if the protein synthesis effect of T3 on injured nerves is a local (at site of injury) or central (at cell body of injured axons).

Methods Employed:

1. Surgical ligation and/or nerve crush of hypoglossal nerve in immature and mature rats.
2. Daily subcutaneous administration of T3 pre-and post-operatively.
3. Transmission Electron Microscopy of the ultrastructural observations of changes in cell body and axons following injury and subsequent administration of T3.
4. Electron staining methods including tannic acid to determine alterations in surface coats of synapses on soma and dendrites of hypoglossal nucleus cells.
5. Freeze fracture techniques of hypoglossal nucleus and nerve to study membrane events associated with nerve injury and T3 administration.
6. Light microscopic examination of thyroid gland to determine the functional state of gland in normal vs T3 animals.
7. Neurophysiological studies to determine the functional regeneration of axons.

Major Findings: This study has just been initiated and therefore no findings are available at this time.

Preliminary experiments are in progress to determine a drug dosage level which ensures maximum protein synthesis without toxicity to the animal over long term administration.

The effects of Triiodothyronine (T3) will be studied in models capable of regeneration as well as those in which regeneration is limited. Studying the effects of T3 on these two different systems affords the opportunity to determine:

- a) if system incapable of regeneration can be switched to a metabolic program which augments the regenerative capacity.
- b) if the increase in protein synthesis produced by T3 is local (at site of injury) or central (at cell body of injured axons).
- c) the basic mechanism of action of T3 on axonal regeneration.

Proposed Course of the Project: To continue as described into the next fiscal year.

Publications: none.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 NS 02096-03 CN
PERIOD COVERED July 1, 1975 through Jan. 30, 1976		
TITLE OF PROJECT (80 characters or less) Effects of reticular stimulation and Metrazol seizures on the electrical and metabolic activity of the cat cortex.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: B. Wern, M.D. OTHER: W.C. Whitehouse W.H. Schuette	Clinical Associate Section head Biomedical Engineer	CN NINCDS PSD CC BEI DRS
COOPERATING UNITS (if any) Biomedical Engineering Branch, Division of Research Services Television Engineering Section, Clinical Center		
LAB/BRANCH Clinical Neurosciences		
SECTION Clinical Neurophysiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2
SUMMARY OF WORK (200 words or less - underline keywords)		
1. Using the <u>fluorescein</u> reference described in the previous report, reliable recordings of cortical <u>NADH fluorescence</u> changes were made. 2. <u>Brain stem stimulation</u> caused a marked elevation of BP and a simultaneous decrease in NADH fluorescence over large cortical areas. The changes in NADH were not always seen, however, during densitometric analysis of smaller <u>cortical regions</u> during video-tape playback. Repeated analyses demonstrated absolutely no consistency in the distribution of anomalous NADH changes. 3. <u>Metrazol seizures</u> were usually accompanied by large decreases in NADH fluorescence over most cortical areas, although small cortical regions manifesting anomalous ictal increases in NADH were occasionally observed. 4. Changes in <u>sagittal sinus flow</u> and cortical 529 mμ fluorescence occurred simultaneously during <u>brain stem stimulation</u> and seizures.		

Project Description:

Objectives: To study the effects of reticulo-cortical activation and of Metrazol seizures on the NADH fluorescence of various cortical areas and on cortical blood flow.

Methods Employed: Adult cats were anesthetized with sodium thiopental or sodium pentobarbital, immobilized with Flaxedil, and artificially ventilated with moisturized 100% O₂. The superior sagittal sinus was cannulated. The exposed cortex was illuminated with UV light and viewed with a television fluorometer. After injection of sodium fluorescein, 529 nM, 460 nM and corrected NADH fluorescence were recorded on videotape, along with arterial blood pressure (BP) and ECoG. Cortical activation was affected either by electrical stimulation of the ponto-mesencephalic junction or by i.v. injection of Metrazol in a dose sufficient to cause spontaneous seizures (100 mg/kg). Sagittal sinus blood flow was continuously monitored through a sinus cannula.

Major Findings:

1. Using the fluorescein reference described in the previous report, reliable recordings of cortical NADH fluorescence changes were made.
2. Brain stem stimulation caused a marked elevation of BP and a simultaneous decrease in NADH fluorescence over large cortical areas. The changes in NADH were not always seen, however, during densitometric analysis of smaller cortical regions during videotape playback. Repeated analyses demonstrated absolutely no consistency in the distribution of anomalous NADH changes.
3. Metrazol seizures were usually accompanied by large decreases in NADH fluorescence over most cortical areas, although small cortical regions manifesting anomalous ictal increases in NADH were occasionally observed.
4. Changes in sagittal sinus flow and cortical 529 nM fluorescence occurred simultaneously during brain stem stimulation and seizures.

Proposed Course of the Project: This project has been completed. Some data are still being assembled for purposes of publication.

Publications:

Vern, B., Whitehouse, W.C., and Schuette, W.H.: Sodium fluorescein: a new reference for NADH fluorometry. Brain Res.: 98: 405-409. 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02121-02 CN
PERIOD COVERED: July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) [K ⁺] _c clearance in epileptogenic glial scars of Alumina cream Ectol on Maca Mulatec		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	D.V. Lewis, M.D. N. Mutsuga, M.D.	Clinical Associate Visiting Fellow Biomedical Engineer Associate Chief
OTHER:	W.H. Schuette J.M. Van Buren, M.D.	CN NINCDs CN NINCDs BEI DRS CN NINCDs
COOPERATING UNITS (if any) Biomedical Engineering Branch, Division of Research Services Television Engineering Section, Clinical Center		
LAB/BRANCH Clinical Neurosciences		
SECTION Clinical Neurophysiology		
INSTITUTE AND LOCATION NINCDs, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.8	OTHER: 0.1
SUMMARY OF WORK (200 words or less - underline keywords) <p> <u>Reactive gliosis</u> at the sites, in which <u>potassium clearance</u> was measured was quantitated histologically and correlated with potassium clearance rates. A small, significant correlation was observed, with dense gliosis associated with slowed potassium clearance, although the baseline potassium level appeared no different in actively <u>epileptogenic areas</u> or gliotic areas compared with normal areas. The results suggest a negative correlation between reactive gliosis and rapidity of extracellular potassium clearance. </p>		

Project Description:

Objectives: To correlate any observed changes in $[K^+]_o$ reuptake with the degree of fibrous astrocytic proliferation in or near the epileptogenic lesions induced by $AL(OH)_3$ injection in the monkey motor cortex.

Methods Employed: $[K^+]_o$ measurement is done as in project No. Z01 NS 02122. $[K^+]_o$ changes are induced by direct cortical stimulation. Brains are perfused, fixed and fibrous astrocytes and neurons are quantitated utilizing standard neuropathological techniques. The observed $[K^+]_o$ kinetics are then correlated with the amount of fibrous astrocytes and/or neurons observed in the region of measured $[K^+]_o$ kinetics.

Major Findings: Reactive gliosis at the sites in which potassium clearance was measured was quantitated histologically and correlated with potassium rates. A small, significant correlation was observed, with dense gliosis associated with slowed potassium clearance, although the baseline potassium level appeared no different in actively epileptogenic areas or gliotic areas compared with normal areas. The results suggest a negative correlation between reactive gliosis and rapidity of extracellular potassium clearance.

Proposed Course of the Project: Project completed. Paper to be submitted for publication.

Publications: none.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02123-02 CN
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) Fluorometric and oxygen consumption changes in the cat cortex		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <div style="display: flex; justify-content: space-between;"> <div> PI: D.V. Lewis, M.D. OTHER: W.H. Schuette </div> <div> Clinical Associate Biomedical Engineer </div> <div> CN NINCDS BEI DRS </div> </div>		
COOPERATING UNITS (if any) Biomedical Engineering Branch, Division of Research Services Television Engineering Section, Clinical Center		
LAB/BRANCH Clinical Neurosciences		
SECTION Clinical Neurophysiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.2	OTHER: 0.1
SUMMARY OF WORK (200 words or less - underline keywords) <p> <u>NADH fluorescence</u> within the cortical area drained by the sinus, could be correlated with <u>oxygen consumption</u> changes calculated from the <u>sinus flow</u> and saturation values. The onset and peak values of calculated oxygen consumption and NADH fluorescence changes usually occurred within several seconds of one another and high, significant correlations were found between the maximum changes in both parameters following stimulation. Also the magnitude of $[K^+]_o$ changes and calculated oxygen consumption changes correlated well. </p>		

Project Description:

Objectives: To correlate oxygen consumption, blood flow and Hgb saturation with NADH fluorescence changes in the cat cortex during direct cortical stimulation. If the NADH fluorescence changes can be correlated with separate on line measures of O_2 consumption this will strengthen and clarify the relationship between fluorometric changes and O_2 consumption rates. In addition, with $[K^+]_o$ (see below) and NADH monitoring along with flow, some information on the local regulation of cerebral blood flow may be obtained.

Methods Employed: Intracellular redox changes as indicated by changes in the ratio of oxidized to reduced nicotine adenine dineucleotide are monitored fluorometrically in the superficial 1 mm of the cat cerebral cortex. Blood flow from the monitored area is measured by a drip meter from the cannulated sagittal sinus. Hgb saturation is measured continuously in the cannula by means of a miniature oximeter.

Major Findings: NADH fluorescence, sagittal sinus blood flow and sinus hemoglobin saturation were monitored simultaneously during cortical stimulation of a wide area of the anterior and mid suprasylvian and marginal gyri. The area monitored fluorometrically was located within the area apparently drained by the sinus, so that the fluorometric changes could be correlated with oxygen consumption. The onset and peak values of calculated oxygen consumption and NADH fluorescence changes usually occurred within several seconds of one another and high, significant ($r > 0.9$ and $P < .01$) correlations were found between the maximum changes in both parameters following stimulation. The relation of cortical $[K^+]_o$ changes to oxygen consumption changes was also explored; again the magnitude of $[K^+]_o$ changes and calculated oxygen consumption changes correlated well. The demonstrated agreement between fluorometric and direct (sinus cannulation) measurements of oxidative metabolism reinforces the interpretation of in situ cortical fluorescence changes as indicative of changes in oxygen consumption rate.

Proposed Course of the Project: Project completed. A paper is in press.

Publications:

Lewis, D.V. and Schuette, W.H.: NADH fluorescence, $[K^+]_o$ and oxygen consumption in cat cerebral cortex during direct cortical stimulation. Brain Res.: in press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02124-02 CN												
PERIOD COVERED July 1, 1975 through June 30, 1976														
TITLE OF PROJECT (80 characters or less) Cortical O ₂ consumption and [K ⁺] _o in relation to NADH oxidation during cortical activation and Metrazol seizures.														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: B. Vern, M.D.</td> <td style="width: 40%;">Clinical Associate</td> <td style="width: 20%;">CN NINCDS</td> </tr> <tr> <td>OTHER: W.H. Schuette</td> <td>Biomedical Engineer</td> <td>BEI DRS</td> </tr> <tr> <td>W.C. Whitehouse</td> <td>Section Head</td> <td>PSD CC</td> </tr> <tr> <td>N. Mutsuga, M.D.</td> <td>Visiting Fellow</td> <td>CN NINCDS</td> </tr> </table>			PI: B. Vern, M.D.	Clinical Associate	CN NINCDS	OTHER: W.H. Schuette	Biomedical Engineer	BEI DRS	W.C. Whitehouse	Section Head	PSD CC	N. Mutsuga, M.D.	Visiting Fellow	CN NINCDS
PI: B. Vern, M.D.	Clinical Associate	CN NINCDS												
OTHER: W.H. Schuette	Biomedical Engineer	BEI DRS												
W.C. Whitehouse	Section Head	PSD CC												
N. Mutsuga, M.D.	Visiting Fellow	CN NINCDS												
COOPERATING UNITS (if any) Biomedical Engineering Branch, Division of Research Services Television Engineering Section, Clinical Center														
LAB/BRANCH Clinical Neurosciences														
SECTION Clinical Neurophysiology														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014														
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.4	OTHER: 0.2												
SUMMARY OF WORK (200 words or less - underline keywords) <p> The time integrals of <u>NADH</u> decreases and <u>cortical O₂ consumption</u> increases during normotensive <u>seizures</u> were linearly related. There was no correlation between NADH oxidation and O₂ consumption during hypotensive seizures. Increases in cortical <u>oxidative metabolism</u> following <u>brain stem</u> stimulation were related to the concomitant increases in <u>blood pressure</u> and SSF rather than to activation of <u>ECoG</u>. <u>Cortical ischemia</u> was characterized by a simultaneous increase in NADH fluorescence and decrease in O₂ consumption. The clearance of <u>extracellular K⁺</u> after the termination of <u>ictal activity</u> was not a monoexponential function. This clearance was slowed by ischemia. </p>														

Project Description:

Objectives:

1. To study the relationship between cortical oxygen consumption and NADH oxidation during cortical activation.
2. To study the effects of ischemia on ictal changes in cortical NADH fluorescence, O_2 consumption, and $[K^+]_o$.

Methods Employed: Cats were anesthetized, paralyzed, and artificially ventilated. After exposure of the cortex, the superior sagittal sinus was cannulated. Sagittal sinus blood flow (SSF) and venous oxygen hemoglobin saturation were monitored, enabling the calculation of relative cortical oxygen consumption. Cortical NADH fluorescence was continuously monitored with a television fluorometer. The cortex was activated either by brain stem stimulation or by i.v. Metrazol injection.

In addition to the methods described in the previous report (02096) cortical $[K^+]_o$ was recorded with K^+ -sensitive micro-electrodes during Metrazol seizures, following bilateral thoracotomy.

In order to induce ischemia during seizures, two methods were employed: lowering of blood pressure and carotid artery occlusion.

Major Findings:

1. The time integrals of NADH decreases and of increases in cortical oxygen consumption during normotensive seizures were linearly related. There was no correlation between NADH oxidation and O_2 consumption during hypotensive seizures.
2. Increases in cortical oxidative metabolism following brain stem stimulation were related to the concomitant increases in blood pressure and SSF rather than to activation of the ECoG.
3. Cortical ischemia was characterized by a simultaneous increase in NADH fluorescence and decrease in O_2 consumption.
4. The clearance of extracellular K^+ after the termination of ictal activity was not a monoexponential function. This clearance was slowed by ischemia. The pattern and behavior of the K^+ clearance curves observed were consistent with a major participation of an O_2 dependent process in the control of elevated $[K^+]_o$. The role of glial cells in this process was suggested.

Proposed Course of the Project: This project is completed. A paper is currently in press and another is in preparation.

Publications:

Vern, B., Schuette, W.H., Whitehouse, W.C. and Mutsuga, N.:
Cortical oxygen consumption and NADH fluorescence during
Metrazol^R seizures in normotensive and hypotensive cats.
Exper. Neurol.: In press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02206-01 CN
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) Contribution of passive diffusion to clearance of extracellular potassium.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: B. Vern, M.D. OTHER: W.H. Schuette L. Thibault	Clinical Associate Biomedical Engineer Biomedical Engineer	CN NINCDS BEI DRS BEI DRS
COOPERATING UNITS (if any) Biomedical Engineering Branch, Division of Research Services		
LAB/BRANCH Clinical Neurosciences		
SECTION Clinical Neurophysiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2
SUMMARY OF WORK (200 words or less - underline keywords) A mathematical model for <u>[K⁺] clearance</u> , incorporating both passive diffusion and active uptake processes, was developed. After specifying the initial conditions (the spatial profiles of <u>[K⁺]</u> at time = 0 following <u>electrical stimulation</u>), the experimental data were incorporated into the mathematical formulation. Passive diffusion probably makes a relatively insignificant contribution to the clearance of elevated <u>[K⁺]</u> in the <u>cortex</u> . Active processes are probably more important in this regard.		

Project Description:

Objectives: To determine the contribution of intra-cortical diffusion to the clearance of elevated $[K^+]_o$ following electrical stimulation.

Methods Employed: Cats were anesthetized (sodium pentobarbital) immobilized (Flaxedil) and artificially respired (100% O_2). The suprasylvian gyri were exposed bilaterally, and dams were constructed with dental cement to allow the creation of a pool of mineral oil over exposed cortex. $[K^+]_o$ was measured (with K^+ -sensitive microelectrodes) at a number of locations at various distances from a monopolar stimulating electrode, along both vertical and horizontal coordinates. The effects of distance from the stimulating electrode on the amplitude and time course of $[K^+]_o$ changes was studied.

Major Findings: A mathematical model for $[K^+]_o$ clearance, incorporating both passive diffusion and active uptake processes, was developed. After specifying the initial conditions (the spatial profiles of $[K^+]_o$ at time = 0 following electrical stimulation), the experimental data were incorporated into the mathematical formulation. Passive diffusion probably makes a relatively insignificant contribution to the clearance of elevated $[K^+]_o$ in the cortex. Active processes are probably more important in this regard.

Proposed Course of the Project: This project is completed. Data are being prepared for publication.

Publications: none.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 201 NS 02207-01 CN
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) NADH fluorescence and $[K^+]_o$ in the gerbil cortex following carotid artery occlusion.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: B. Vern, M.D. OTHER: N. Mutsuga, M.D. W.C. Whitehouse	Clinical Associate Visiting Fellow Section Head	CN NINCDS CN NINCDS PSD CC
COOPERATING UNITS (if any) Television Engineering Section, Clinical Center		
LAB/BRANCH Clinical Neurosciences		
SECTION Clinical Neurophysiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2
SUMMARY OF WORK (200 words or less - underline keywords) Unilateral <u>carotid occlusion</u> caused an ipsilateral increase in <u>NADH fluorescence</u> which was usually reversible if the occlusion was not maintained longer than about 1 min. Stable $[K^+]_o$ recordings were difficult to obtain because of the susceptibility of the <u>gerbil cortex</u> to <u>spreading depression</u> .		

Project Description:

Objectives: To detect possibly lateralized alterations of cortical oxidative metabolism during unilateral carotid artery occlusion.

Methods Employed: Mongolian gerbils were anesthetized with intraperitoneal pentobarbital. Surgical ties were loosely placed around either one or both common carotid arteries to enable rapid and reversible occlusion of these vessels. Following removal of the calvarium, the cortex was illuminated with UV light and viewed with a television fluorometer. Sodium fluorescein was used as a fluorometric reference. $[K^+]_o$ was monitored from a depth of 500 μ in the cortex with K^+ -sensitive microelectrodes. One or both carotids were occluded for periods between 5 sec and 5 min.

Major Findings: Unilateral carotid occlusion caused an ipsilateral increase in NADH fluorescence which was usually reversible if the occlusion was not maintained longer than about 1 min. Stable $[K^+]_o$ recordings were difficult to obtain because of the susceptibility of the gerbil cortex to spreading depression.

Proposed Course of the Project: The above results are preliminary. More attention will be paid to maintaining the cortex in a normal physiological state, thus hopefully avoiding the problem of spreading depression. The effect of time of occlusion on the reversibility of NADH changes will be reinvestigated.

Publications: none.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02208-01 CN												
PERIOD COVERED: July 1, 1975 through June 30, 1976														
TITLE OF PROJECT (80 characters or less) Relationship between cortical blood flow and 529 nM fluorescence														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0"> <tr> <td>PI:</td> <td>B. Vern, M.D.</td> <td>Clinical Associate</td> <td>CN NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>W.H. Schuette</td> <td>Biomedical Engineer</td> <td>BEI DRS</td> </tr> <tr> <td></td> <td>W.C. Whitehouse</td> <td>Section Head</td> <td>PSD CC</td> </tr> </table>			PI:	B. Vern, M.D.	Clinical Associate	CN NINCDS	OTHER:	W.H. Schuette	Biomedical Engineer	BEI DRS		W.C. Whitehouse	Section Head	PSD CC
PI:	B. Vern, M.D.	Clinical Associate	CN NINCDS											
OTHER:	W.H. Schuette	Biomedical Engineer	BEI DRS											
	W.C. Whitehouse	Section Head	PSD CC											
COOPERATING UNITS (if any) Biomedical Engineering Branch, Division of Research Services Television Engineering Branch, Clinical Center														
LAB/BRANCH Clinical Neurosciences														
SECTION Clinical Neurophysiology														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014														
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2												
SUMMARY OF WORK (200 words or less - underline keywords) Preliminary analysis indicates that <u>sagittal sinus blood flow</u> changes are related to the product of the <u>blood pressure</u> and the square of changes in <u>529 nM fluorescence</u> intensity in most <u>cortical areas</u> which are probably within the sagittal sinus drainage bed.														

Project Description:

Objectives: To define a semiquantitative relationship between cortical blood flow and the fluorescence of i.v. injected sodium fluorescein.

Methods Employed: The cortex was widely exposed in anesthetized, paralyzed, artificially ventilated cats. Ventilation rate was adjusted to give an end-expiratory $[CO_2]$ of between 3.5 and 4.5%. The cortex was illuminated with UV light and viewed with a television fluorometer. Cortical 529 nM fluorescence was monitored after the i.v. injection of 3-5cc of 1% sodium fluorescein. Sagittal sinus blood flow (SSF) was monitored with a drip counter/integrator attached to a sagittal sinus cannula. BP was monitored through a femoral arterial cannula. Changes in SSF were induced by either Metrazol seizures or by pharmacologic alterations of the BP. Regional cortical changes in 529 nM fluorescence were determined by video-densitometry.

Major Findings: Preliminary analysis indicates the SSF changes are related to the product of the blood pressure and the square of changes in 529 nM fluorescence intensity in most cortical areas which are probably within the sagittal sinus drainage bed.

Proposed Course of the Project: The validity of the $(BP \times 529 \text{ nM fluorescence change})$ calculation will be further investigated. Cortical vascular dilation will be induced with 5% CO_2 inhalation.

Publications: none.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02209-01 CN									
PERIOD COVERED July 1, 1975 through June 30, 1976											
TITLE OF PROJECT (80 characters or less) NADH fluorometry of the <u>in vivo</u> cat heart during acute coronary occlusion.											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: B. Vern, M.D.</td> <td style="width: 33%;">Clinical Associate</td> <td style="width: 33%;">CN NINCDS</td> </tr> <tr> <td>OTHER: W.H. Schuette</td> <td>Biomedical Engineer</td> <td>BEI DRS</td> </tr> <tr> <td>W.C. Whitehouse</td> <td>Section Head</td> <td>PSD CC</td> </tr> </table>			PI: B. Vern, M.D.	Clinical Associate	CN NINCDS	OTHER: W.H. Schuette	Biomedical Engineer	BEI DRS	W.C. Whitehouse	Section Head	PSD CC
PI: B. Vern, M.D.	Clinical Associate	CN NINCDS									
OTHER: W.H. Schuette	Biomedical Engineer	BEI DRS									
W.C. Whitehouse	Section Head	PSD CC									
COOPERATING UNITS (if any) Biomedical Engineering Branch, Division of Research Services Television Engineering Section, Clinical Center											
LAB/BRANCH Clinical Neurosciences											
SECTION Clinical Neurophysiology											
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014											
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1									
SUMMARY OF WORK (200 words or less - underline keywords) <p> <u>Fluorescein</u> injection following acute <u>coronary occlusion</u> caused the ischemic area of <u>myocardium</u> to appear dark against brightly fluorescent surrounding tissue. The border between normal and ischemic tissue was always clearly defined. Coronary occlusion always caused an increase in <u>NADH fluorescence</u> in the ischemic areas. Simultaneous changes in <u>529 nM fluorescence</u> in these areas suggested an increase in the blood volume of acutely "ischemic" myocardium. These techniques may have a broad applicability in human <u>coronary vascular surgery</u>, e.g., to evaluate the effectiveness of coronary bypass grafts. </p>											

Project Description:

Objectives: To record changes in NADH fluorescence of the intact exposed myocardium during acute occlusion of a coronary artery.

Methods Employed: Cats were anesthetized with barbiturate, immobilized with Flaxedil, and respired with 100% O₂. The anterior surface of the myocardium was exposed through a mid-sternal approach. Arterial BP was monitored and used to assess the level of anesthesia. A silk suture was passed around either the entire left anterior descending coronary artery or one of its major branches in such a way as to allow rapid and reversible vascular occlusion. The heart was then viewed with a television fluorometer. NADH fluorescence was measured using sodium fluorescein fluorescence as a reference. The areas of ischemia were visualized by i.v. injections of fluorescein immediately following coronary occlusion.

Major Findings: Fluorescein injection following acute coronary occlusion caused the ischemic area of myocardium to appear dark against brightly fluorescent surrounding tissue. The border between normal and ischemic tissue was always clearly defined. Coronary occlusion always caused an increase in NADH fluorescence in the ischemic areas. Simultaneous changes in 529 nM fluorescence in these areas suggested an increase in the blood volume of acutely "ischemic" myocardium. These techniques may have a broad applicability in human coronary vascular surgery, e.g., to evaluate the effectiveness of coronary bypass grafts..

Proposed Course of the Project: This project is completed. A manuscript is being prepared.

Publications: none.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02210-01 CN
PERIOD COVERED: July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) GABA antagonism by Penicillin in Somatosensory Cortex		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: J.B. Macon, M.D. Clinical Associate CN NINCDS		
COOPERATING UNITS (if any)		
none		
LAB/BRANCH Clinical Neurosciences		
SECTION Clinical Neurophysiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.5	OTHER: 0.2
SUMMARY OF WORK (200 words or less - underline keywords)		
<p><u>GABA iontophoresis</u> produced inhibitory effects on the firing rate of all <u>somatosensory cortex</u> neurons encountered. Other amino acids including <u>glycine</u>, <u>B-alanine</u>, and <u>taurine</u> had less potent inhibitory effects. All neurons were excited by <u>glutamate</u> or <u>d,l-homocysteic acid</u>. <u>Penicillin iontophoresis</u> produced an increase in spontaneous firing rate of most units studied. GABA inhibition is weakly antagonized by iontophoretic currents greater than 100 nA of penicillin. GABA <u>receptor</u> antagonism is unlikely to be the primary mechanism by which penicillin produces acute <u>epileptic foci</u>.</p>		

Project Description:

Objectives: To determine if gamma amino butyric acid (GABA) receptor antagonism plays a role in the evolution of acute penicillin epileptic foci in feline somatosensory cortex.

Methods Employed: Extracellular single unit activity in cat somatosensory cortex was studied via one barrel of a compound seven-barrelled micropipette. Penicillin and putative neurotransmitter agents were delivered by iontophoresis from the other six barrels. The recording electrode protruded 40-50 μ distal to a five-barrel pipette containing neurotransmitter agents. A penicillin electrode was placed 40-50 μ proximal to the five-barrel electrode. The compound micropipette was placed in the postcruciate or coronal gyri of the cat cortex under visual control. The cat was anesthetized lightly with Nembutal and paralyzed with Flaxedil and artificially respired. In some experiments penicillin was applied topically by a small pledget rather by iontophoresis.

Major Findings: GABA iontophoresis produced inhibitory effects on the firing rate of all somatosensory cortex neurons encountered. Other amino acids including glycine, B-alanine, and taurine had less potent inhibitory effects. All neurons were excited by glutamate or d, l-homocysteic acid.

Penicillin iontophoresis produced an increase in spontaneous firing rate of most units studied. Enhanced evoked responses and increased receptive field size occurred in most cases. During penicillin iontophoresis initial studies indicate that GABA inhibition is weakly antagonized by iontophoretic currents greater than 100 nA of penicillin.

Single units firing in bursts as part of a topical penicillin focus exhibited an increased background activity between bursts when glutamate was iontophoresed. GABA iontophoresis to such units eliminated this background activity without inhibiting the unit bursts which were occurring simultaneously with surface interictal spikes.

Preliminary results indicate that penicillin applied directly to a cortical neuron may weakly antagonize GABA receptors, but that penicillin topical foci contain neurons with intact GABA receptors. This suggests that GABA receptor antagonism is unlikely to be the primary mechanism by which penicillin produces acute epileptic foci.

Proposed Course of the Project: To continue throughout the next fiscal year. Plan to investigate possible antagonism by penicillin agents and to compare with other epileptogenic agents

Serial No. Z01 NS 02210-01 CN

including bicuculline, strychnine, and picrotoxin.

Publications: none.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02211-01 CN
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) Deafferentation Hyperactivity in the Spinal Trigeminal Nucleus		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: J.B. Macon, M.D. Clinical Associate CN NINCDS		
COOPERATING UNITS (if any) Experimental Surgery Unit, Veterinary Resources Branch, Division of Research Services		
LAB/BRANCH Clinical Neurosciences		
SECTION Clinical Neurophysiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.5	OTHER: 0.2
SUMMARY OF WORK (200 words or less - underline keywords) To determine if <u>deafferentation</u> plays a role in the development of <u>epileptiform-like activity</u> in subcortical structures. Eleven cats have undergone successful retrogasserian <u>trigeminal rhizotomy</u> to date. Two normal cats have been studied to provide control responses. Neurons of the normal spinal trigeminal nucleus were found to have low spontaneous rates and strong evoked responses from the appropriate receptive fields. Latency to skin stimulation in n. interpolaris or caudalis ranged from 2-5 msec. Neurons were inhibited by <u>GABA</u> , <u>B-alanine</u> , and <u>glycine</u> . Neurons were excited by <u>glutamate</u> and <u>d,l-homocysteic acid</u> .		

Project Description:

Objectives: To investigate the mechanism of increased spontaneous activity of neuronal firing in the feline spinal trigeminal nucleus following retrogasserian trigeminal rhizotomy. To determine if deafferentation plays a role in the development of epileptiform-like activity in subcortical structures.

Methods Employed: Six-barrelled compound micropipettes were used to record extracellular single unit activity in the cat spinal trigeminal nucleus while iontophoresing putative neurotransmitter agents. Unilateral retrogasserian trigeminal rhizotomies were performed by the subtemporal approach 2 weeks - 3 months prior to recording. The contralateral spinal nucleus was used as control to compare results with deafferented neurons of the ipsilateral side.

Major Findings: Eleven cats have undergone successful retrogasserian trigeminal rhizotomy to date. Two normal cats have been studied to provide control responses. Neurons of the normal spinal trigeminal nucleus were found to have low spontaneous rates and strong evoked responses from the appropriate receptive fields. Latency to skin stimulation in n. interpolaris or caudalis ranged from 2-5 msec. Neurons were inhibited by GABA, B-alanine, and glycine. Neurons were excited by glutamate and d, l-homocysteic acid.

Proposed Course of Project: To continue throughout the next fiscal year. Plan to study the sensitivity of deafferented neurons to iontophoretically applied neurotransmitters 2 weeks - 3 months post rhizotomy. Attempt to determine whether there is a modification of receptor sensitivity in the deafferented neuron to account for the observed spontaneous hyperactivity.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02212-01 CN
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) Clinical ictal patterns in epileptics with EEG temporal lobe foci		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: C. Ajmone Marsan, M.D. Chief CN NINCDS D.W. King, M.D. Clinical Associate CN NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Neurosciences		
SECTION Clinical Neurophysiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.7	OTHER:
SUMMARY OF WORK (200 words or less - underline keywords) This study utilizes over 200 patients. These are selected from the files of our laboratory on the basis of a) presence of <u>EEG epileptiform activity</u> localized within one or both temporal regions; b) availability of reliable and detailed repeated observations and description of seizure episodes. This project is expected to yield data which may throw some light on the pathophysiology of <u>temporal lobe seizures</u> . From a practical viewpoint the study should provide useful information for a more accurate <u>localization</u> of the <u>epileptogenic process</u> and a better selection of candidates for <u>surgical treatment</u> .		

Project Description:

Objectives: The ictal clinical manifestations of "temporal" epilepsy are extremely complex and variable in different patients or in the same subject at different times. This project is an attempt to analyze in detail such manifestations and to correlate them with the actual localization of the inter-ictal electro-graphic signs.

Methods Employed and Major Findings: This study utilizes over 200 patients. These are selected from the files of our laboratory on the basis of a) presence of EEG epileptiform activity localized within one or both temporal regions; b) availability of reliable and detailed repeated observations and description of seizure episodes. The study is still at the stage of data collection and tabulation: no major findings are available.

Significance: This project is expected to yield interesting data which may throw some light on the pathophysiology of temporal lobe seizures. From a practical viewpoint the study should provide useful information for a more accurate localization of the epileptogenic process and a better selection of possible candidates for surgical treatment, this helping in the management of the main seizure disorder.

Proposed Course of the Project: This project should be completed within the next fiscal period.

Publications: none.

ANNUAL REPORT
July 1, 1975 through June 30, 1976
Developmental and Metabolic Neurology Branch
National Institute of Neurological and Communicative Disorders and Stroke
Roscoe O. Brady, Chief

The Branch has continued to be one of the foremost research units in the world with regard to contributions to the control and therapy of disorders of the nervous system. Many of the findings in the immediate past year and in previous years have had direct application for the treatment of patients, diagnosis of heritable diseases, and genetic counseling for inherited metabolic disorders. Insight has been obtained into the control of a newly identified form of inherited metabolic diseases, namely conditions caused by deficiencies of synthetic enzymes. Other work deals with specific pathogenetic aspects of neoplastic diseases, multiple sclerosis, a novel heritable triad associated with mental retardation, mucopolysaccharidoses, and factors that predispose younger individuals to stroke. Because these investigations cover a wide but related base for the control and therapy of a number of human disorders, this report is divided into the following relevant categories:

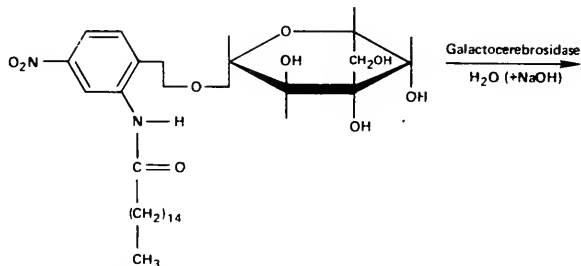
I. Enzyme Replacement Therapy for Inherited Diseases

During the past year we have established the efficacy of exogenous glucocerebrosidase in vivo. This enzyme is lacking in patients with Gaucher's disease. Thus, for every unit of enzyme activity injected (a unit catalyzes the hydrolysis of 1 nanomole of glucocerebroside per hour) we find that 0.4 nanomole of glucocerebroside has been cleared from the liver in each of three patients infused with glucocerebrosidase. This efficiency is very encouraging since it is obvious that a considerable amount of the enzyme is taken up by tissues other than liver, and in order for the enzyme to exert its physiological effect, it must be pinocytized by the liver cell, packaged into intracellular lysosomes, and be brought into effective contact with the accumulating lipid. That the body carries out all of these operations with 40% efficiency augurs well for enzyme replacement therapy for heritable metabolic diseases.

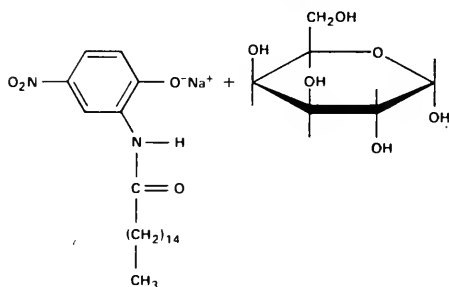
Another discovery of potentially great importance for enzyme replacement therapy was made in the past year in the course of investigating infusion of enzymes into monkeys, rats, and rabbits. It was observed that the injected enzyme persisted in the liver of the injected animal very much longer if the animal were anaesthetized continuously. The enzyme was shown to have been taken up by the liver cell, but it was protected from catabolism and loss of activity by various agents such as pentobarbital and Tilerone. For example, the half-life of glucocerebrosidase in the liver of the control animals is 6 hours, whereas it is greater than 30 hours in animals sedated with pentobarbital. It must be determined whether the surviving enzyme is functioning during this extended period of time. If it is catalytically active, this observation will undoubtedly play a major role in devising strategies of enzyme replacement.

II. Synthesis of Diagnostic Reagents

We have had a major breakthrough in the development of a chromogenic reagent for the diagnosis of Krabbe's globoid cell leukodystrophy. We have synthesized a chromogenic analogue of galactocerebroside, 2-hexadecanoylamino-4-nitrophenyl- β -D-galactopyranoside, that has been shown to be a reliable reagent for the diagnosis of Krabbe's disease and the identification of heterozygous carriers of this disorder. The reactions involved demonstrated in the following figure:



2-Hexadecanoylamino-4-Nitrophenyl- β -D-Galactopyranoside



(Yellow Product)

Galactose

Previously, the diagnosis of these patients, detection of carriers and the monitoring of pregnancies at risk for this disorder required the use of radioactive galactocerebroside. These determinations were therefore restricted to a few research laboratories. The assay with the newly synthesized analogue is so facile that general clinical chemistry laboratories can now perform these tests.

III. Roles of Gangliosides as Cell Receptors, Alterations in Malignant Transformation and Heritable Disorders of Anabolism.

A major breakthrough has occurred in our understanding of the function of acidic glycolipids known as gangliosides. In collaboration with the Section on Biochemistry of Cell Regulation, LBP, NIAMDD, we have demonstrated that one of the functions of gangliosides is to act as cell surface receptors for trophic hormones. In particular, we have shown that thyrotrophic hormone reacts very strongly with ganglioside G_{D1b} . Thyroid membranes contain this ganglioside, and it seems very likely that it (or a glycoprotein with exactly the same oligosaccharide configuration) is the natural receptor for the trophic hormone. Substantiation for this assertion was found in subsequent experiments with rat thyroid tumor cells which lack this ganglioside. These cells do not respond to thyrotropin. Thyrotropin is a glycoprotein whose amino acid sequence is completely established. Furthermore, it has been shown that the amino acid sequences of luteinizing hormone, human chorionic gonadotropin, and cholera toxin have homology with thyrotropin. It is therefore very likely that the effects of these glycopeptides are also mediated by gangliosides on cell membranes. This fact has already been established for cholera toxin where it was shown that the uptake of as little as 17,000 molecules of ganglioside G_{M1} per cell can confer sensitivity to cholera toxin to cells previously unresponsive to this glycopeptide. The latter experiments were carried out in collaboration with the Laboratory of Cellular Metabolism, NHL&BI. These important observations provide significant insight into the mechanism of action of trophic hormones.

In the annual report for FY 1974, we documented the first known case of an inherited defect of ganglioside biosynthesis. We demonstrated that the metabolic lesion in the propositus was exactly the opposite of Tay-Sachs disease; namely an inability to synthesize ganglioside G_{M2} due to a deficiency of an anabolic enzyme rather than an insufficiency of a catabolic enzyme as seen in all the lipid storage diseases. We have recently discovered a second patient with exactly the same phenotypic abnormalities as the index case and we are confident that an identical anabolic enzymatic defect will be demonstrated in this infant. Furthermore, we shall undertake a comprehensive examination concerning the possibility that the maternal X-chromosome carries a DNA virus such as SV 40 or polyoma virus. These viruses are tumorigenic in rodents and their presence causes exactly the same metabolic defect when cells are transformed by these agents.

IV. Myelination

We have isolated myelin-specific glycoproteins from the central and peripheral nervous systems. The carbohydrate composition has been determined and the major components have been shown to be fucose, mannose, galactose, N-acetylglucosamine and N-acetylneuraminic acid. The glycoproteins are highly sulfated indicating an important functional aspect of their con-

stitution; i.e., they are very acid compounds like the mucopolysaccharides.

The glycoprotein in the central nervous system has been shown to be a surface component of myelin. Hence, it is potentially susceptible to attachment of viruses and modification by virus-associated enzymes. The physiological significance of such changes is not completely understood at this time but is certainly conceivable that alterations in the composition of myelin glycoproteins could cause structural and immunological abnormalities. Changes such as these have long been thought to be involved in the pathogenesis of myelin-destructive diseases such as multiple sclerosis.

V. Epilepsy

Metabolic profiles of anti-epileptic drugs were monitored in a number of epileptic patients. It was found that phenobarbital is hydroxylated in the para position of the benzene ring and that much of the derivative appears in the urine conjugated with glucuronic acid. However, the data indicate that most of administered phenobarbital is eliminated by man in the form of still unidentified metabolites.

Studies with epileptic patients indicate that vigilance and reaction times are the most useful parameters in evaluating the psychological effects of anti-epileptic drugs. These determinations may be helpful for evaluating the borderline between therapeutic and toxic levels of anti-epileptic drugs. Regimens for optimal seizure control and functional efficiency may now be better quantitated. Thus, the management of seizure patients is now amenable to more precise evaluation and more effective control of epilepsy should become available.

VI. Mental Retardation

A study has revealed a heritable condition associated with brain damage that appears to be a novel syndrome that is transmitted as an autosomal dominant trait. The symptoms comprise a triad of (1) progressive mental retardation with onset between 12 and 30 years of age; (2) incoordination and slow eye pursuit without rapid saccades or nystagmus; and (3) an associated nevus of Ota in the last generation in each family. Death generally occurred prior to 45 years of age. Pathological findings revealed degeneration of nuclear groups in the upper brainstem and a moderate degree of cortical atrophy. The etiology of this unique condition remains to be classified. Its identification should serve as a point of departure for the elucidation of developmental abnormalities having a common molecular basis.

VII. Mucopolysaccharidoses

We have characterized the mucopolysaccharides in the urine of patients with the Maroteaux-Lamy syndrome. These glycosaminoglycans consist primarily of dermatan sulfate (80%) and chondrotin-4-sulfate. The molecular size of the excreted mucopolysaccharides presented a consistent pattern which will be useful for the diagnosis of this syndrome and for monitoring enzyme replacement trials with sulfatase B which will be undertaken for the treatment of patients with this condition.

VIII. Stroke

A significant proportion (22 out of 171) of young patients with strokes were found to have hypercholesterolemia and/or hypertriglyceridemia. The familial preponderance of these conditions has been demonstrated. The predisposing factors to stroke in young individuals will be further evaluated and the possibility of dietary modification of these conditions will be investigated. These observations will be coupled with our studies on the therapy of Fabry's disease where patients are known to have a predisposition to strokes. The effect of enzyme replacement therapy in this disorder will be investigated as a model for the treatment of cerebrovascular disease.

IX. Award

The Branch was honored in FY 76 by the selection of the Chief of the Branch for the distinguished Achievement Award by the Editors of Modern Medicine.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 201 NS 00706-17 DMN
PERIOD COVERED: July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Inborn errors of metabolism, cerebral degeneration and brain dysfunction of diverse etiology.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Anatole S. Dekaban, M.D., Ph.D., Associate Chief, DMN, NINCDS		
OTHER: George Constantopoulos, Ph.D., Biochemist, DMN, NINCDS Michael P. Whyte, M.D., Clinical Associate, DMN, NINCDS Norio Sakuragawa, M.D., Visiting Fellow, DMN, NINCDS Jan K. Steusing, Research Associate, DMN, NINCDS		
COOPERATING UNITS (if any)		
None		
LAB/BRANCH		
Developmental and Metabolic Neurology Branch		
SECTION		
Clinical Investigations and Therapeutics		
INSTITUTE AND LOCATION		
NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.9	0.1
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> Project No. 201 NS 01523-08 DMN was incorporated in this project. Because of its scope, this is a long range study. According to conservative estimates for this country, over four million people are permanently handicapped by <u>mental retardation</u> (frequently of familial type), <u>birth defects</u>, <u>progressive cerebral degeneration</u> and <u>inborn errors of metabolism</u>. The principal aim is to study the pathogenesis and etiology of these diverse disorders, to identify heterozygotes, and to institute therapeutic modification of the course of the respective diseases. The methodological approach includes clinical and genetic appraisal, <u>tissue culture</u>, <u>enzymology</u>, <u>pathology</u> and <u>chemistry</u> of <u>lipidoses</u> and <u>mucopolysaccharidoses</u>. </p>		

Project Description:

Objectives: Since the majority of chronic neurological disorders affect patients from their early years of life, they constitute a formidable medical and social problem of our times. Pooled together, mental retardation (frequently familial) birth defects, cerebral degenerations and inborn errors of metabolism affecting the nervous system amount to over four million people in the U.S.A. Our main objectives are: 1) to study the pathogenesis and etiology of their diverse disorders which are frequently of genetic origin, 2) to devise special diagnostic tests including identification of heterozygotes, 3) to institute therapeutic modifications of the course of the respective disorders, 4) explore preventive measures such as eugenics and prenatal diagnosis.

Patient Material: Total of 17 inpatients and 25 outpatients were studied. Patients with the following disease categories were admitted for investigation in this order of frequency; mucopolysaccharidoses, sphingolipidoses, ceroid lipofuscinosis, various somatic hereditary syndromes and familial mental retardation.

Methods Employed:

- 1) Comprehensive neurological, developmental and genetic assessment of the patients studied including family study when appropriate.
- 2) Determination of profiles of lipids, amino acids, proteins, mucopolysaccharides and carbohydrates in blood, and when appropriate, in urine and cerebrospinal fluid.
- 3) Assay of enzyme activities in the peripheral blood leukocytes of genetic diseases studied; also, preparation of the karyotypes using banding technique.
- 4) Establishment of skin fibroblast tissue cultures in patients with genetic disorders for study of enzyme activity and turnover studies using radioactive substances. In the case of certain sphingolipidoses and mucopolysaccharidoses, radioactively labelled substrates or elements are added to respective tissue cultures. In the first instance the catabolism, in the second, synthesis of the respective involved substances are followed.
- 5) Employment of invasive techniques, if required, for definitive diagnosis. Brain and liver biopsies are performed and the tissues are used for biochemical, chemical, enzymatic and electron microscopic studies.
- 6) Therapeutic modification of the diseases is attempted whenever possible. For this purpose we use pharmaceuticals, hormones, plasma or formed blood elements, transfusion, dietary modifications and others.
- 7) In case the patient with a metabolic disease dies, samples of the organs and other tissues are stored frozen for future chemical and enzymatic studies; the fresh specimens of tissue are immediately fixed or processed for histochemical and electron microscopic studies.

Major Findings:

- 1) Comprehensive and frequently pioneering studies were conducted in various types of mucopolysaccharidoses (MPS) types I-VI. Multidisciplinary approach was used encompassing detailed clinical, pathological, chemical and enzymatic studies. Peripheral blood components and fibroblast tissue cultures were utilized for determination of enzyme activities. Here, the accomplishments of our biochemist, Dr. Constantopoulos are particularly noteworthy. Using analytical methods as well as a spectrum of catabolic enzymes, he determined in detail for the first time the composition of mucopolysaccharides in different organs of the patients who died with the diagnosis of MPS type III A and MPS type V and extended the knowledge of chemistry in MPS types I and II.

In addition, therapeutic modification studies were conducted in selected patients with MPS type III, V and VI, using long-range administration of corticosteroids (alternate day dosages). The corticosteroid administration was associated with improvement of joint mobility and temporary slight amelioration of mental performance and behavior. The metabolic changes in mucopolysaccharides was evaluated by determination of polymeric and break-down products of urinary mucopolysaccharides accompanied administration of corticosteroids. In general, most of these patients showed a short lasting initial increase in the urinary mucopolysaccharides which was then followed by a sustained reduction. Also, variable changes in ratios of dermatan sulfate to heparan sulfate took place in the patients with MPS types II and V.

- 2) Concentrated efforts were made to introduce therapeutic modifications of selected inborn errors of metabolism. A measure of success was obtained in treatment of kinky hair disease with a new method of parenteral administration of copper, and guiding of chelating dosages of penicillamine of Wilson's disease by repeated metabolic balance studies of copper when both low copper diet and penicillamine were used. The intravenous infusion of isolated enzymes: trihexosidase and glucocerebrosidase to the patients with Fabry's and Gaucher's diseases and their effects on the excessively stored material in various tissues were described in detail in the project No. Z01 NS 00815-16 DMN.
- 3) A study of megalencephaly i.e., of patients with abnormally large brains were completed and new classification and definitions offered. The condition was found to be heterogeneous even after neoplasia were excluded. Among 31 patients tabulated, an abnormally large head (exceeding 2 1/2 standard deviations for the age) was first noted under 1 year of age in 77 per cent of cases. Mental retardation and neurological abnormalities were encountered in 58 per cent and abnormalities other than neurological were present in

22.5 per cent of cases. In those patients who died and autopsy was performed, congenital malformations were the most common abnormality, accounting for two-thirds of cases. The type of malformation varied from minor cytoarchitectonic imperfections to severe arhinencephaly and heterotopias with defective formation of gyri. Thus, the teratogenetic period of the malformation ranged between 4 weeks and over 7 months of gestation. Several presumptive etiological factors were evaluated. The finding, that the condition was infrequently present in several members of the same family, some of whom had normal intelligenc, makes this condition even more interesting; additional study is warranted.

- 4) A study of a family with what appears to be a new syndrome was made. Dominant transmission of an abnormal trait was traced through three consecutive generations. The triad abnormalities consisted of the following triad: progressive mental deterioration with age of onset between 13 and 30 years, incoordination and slow eye pursuit without rapid saccades or nystagmus, an associated finding in the last generation was presence of the nevus of Ota. Death generally occurred prior to the age of 45 years. Degeneration of nuclear groups predominantly in the upper brainstem and moderate degree of cortical atrophy was found at autopsy of one affected person.

Significance to Bio-Medical Research and the Program of the Institute:

Since the majority of infections affecting man are now under quite satisfactory control, the time has come for increased attention to accord a measure of control to such common disorders as hereditary diseases, congenital malformations, mental retardation and degenerative conditions affecting the nervous system. Improved methodology makes it now feasible to advance our knowledge and institute some control in certain of the crippling chronic disorders. These include prenatal diagnosis, enzyme infusions, dietary modifications and institution of certain eugenic measures. Since many of the disorders affect exclusively or predominantly the nervous system the study of etiology and pathogenesis as well as institution of therapeutic trials are of importance in furthering the main mission of our Institute.

Proposed Course of the Project: This is a broad and long range project of our Section. During the next years increasing emphasis will be given to the underlying genetic mechanisms of the hereditary diseases and to therapeutic modifications of respective disorders.

Publications:

1. Dekaban, A.S. and Sakuragawa, N.: Megalencephaly. Chapter in Handbook of Clinical Neurology (in press).
2. Dekaban, A.S. and Brady, R.O.: Therapeutic approaches to selected disorders of inborn errors of metabolism with neurolgical involvement. Int. J. Neurology (in press).

3. Whyte, M. and Dekaban, A.S.: Familial cerebral degeneration with slow eye movements, mental retardation and incidental nevus of Ota. Child Neurol. and Developm. Med. (in press).
4. Dekaban, A.S.: Inborn errors of copper metabolism: Kinky hair disease and hepatolenticular degeneration. Materia Medica Polna (in press).
5. Constantopoulos, G., McComb, R.D. and Dekaban, A.S.: Neurochemistry of the Mucopolysaccharidoses: Brain glycosaminoglycans in normals and four types of mucopolysaccharidoses. J. of Neurochem., (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (DO NOT LEAVE BLANK)	U.S. DEPARTMENT OF PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 00815-16 DMN
PERIOD COVERED July 1, 1975 - June 30, 1976		
TITLE OF PROJECT (80 characters or less) Metabolism of Complex Lipids of Nervous Tissue.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: R. O. Brady, Chief, Dev. & Metab. Neurol. Br., DMN, NINCDS OTHER: P. G. Pentchev, Biochemist, DMN, NINCDS A. E. Gal, Organic Chemist, DMN, NINCDS J. W. Kusiak, Staff Fellow, DMN, NINCDS F. S. Furbish, Guest Worker, DMN, NINCDS		
COOPERATING UNITS (if any) Weizmann Institute of Science, Rehovot, Israel Tufts University Medical School, Boston, Massachusetts		
LAB/BRANCH Developmental & Metabolic Neurology Branch		
SECTION Enzymology and Genetics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 6.8	PROFESSIONAL: 4.8	OTHER: 2.0
SUMMARY OF WORK (200 words or less - underline keywords) Procedures are developed for the purification of enzymes from tissues such as human placenta that are lacking in patients with <u>Gaucher's disease</u> , <u>Fabry's disease</u> , <u>Tay-Sachs disease</u> and <u>Niemann-Pick disease</u> . The effects of <u>enzyme replacement therapy</u> in patients with these disorders is under investigation. Procedures are developed for the <u>diagnosis</u> of patients with these disorders, the <u>detection of heterozygous carriers</u> of these genetic traits, and for the <u>monitoring of pregnancies at risk</u> for each of these diseases.		

Project Description:

Objectives: (1) To elucidate the biosynthetic pathways for the formation of long chain fatty acids, cerebroside, gangliosides, and sphingomyelin; (2) to study the control mechanisms which regulate these processes; (3) to study the metabolic fate of sphingolipids in normal and lipodystrophic disease states, and (4) to provide diagnostic and therapeutic procedures for the amelioration and control of the lipid storage diseases.

Methods: Glucocerebroside and galactocerebroside labeled with ^{14}C in either the hexose or fatty acid portion of the molecule have been synthesized. ^{14}C -labeled sphingomyelin and gluco- and galactopsychosine have been similarly prepared. Ceramide-trihexoside and ceramide tetrahexoside (globoside) specifically labeled with radioactive hydrogen- ^3H have been prepared. The metabolism of these labeled materials has been investigated in vivo and in vitro. Human placenta has proved to be a convenient and rich source of sphingolipid hydrolases. Isolation of these enzymes for therapeutic replacement trials is a major continuing portion of this project.

Major Findings: (1) We have previously reported that enzyme replacement in Gaucher's disease and Fabry's disease promises to be effective therapeutic procedures for the amelioration of these disorders. A long lasting reduction of blood glucocerebroside, the accumulating lipid in Gaucher's disease was observed in two of three patients infused with purified human placental glucocerebrosidase. Patients with Gaucher's disease have subnormal activity of this enzyme in their tissues. The lack of change in blood glucocerebroside in the third recipient is most likely due to the fact that only 8% of the accumulated lipid was cleared from the liver in this patient whereas there was a 26% reduction in accumulated in liver glucocerebroside in the first two patients. The discrepancy in the percentage cleared was due to the fact that the third patient had 24 times more glucocerebroside in her liver than the first and 11 times more than the second. An observation that is of particular importance was that in all three recipients, a constant amount of glucocerebroside was cleared per unit of enzyme injected. Thus, we now know precisely how effective the exogenous glucocerebrosidase is in vivo. Therefore, we can estimate how much enzyme will be required by Gaucher patients by determining the amount of glucocerebroside in a liver biopsy specimen obtained prior to the enzyme replacement trial.

(2) We have found that sedation of animals injected with human placental glucocerebrosidase dramatically prolongs the presence of the exogenous enzyme in the animal tissues. This observation has great potential importance for enzyme replacement in humans if it can be shown that the exogenous enzyme is catalytically active over the extended period of time. We are currently devising experiments to provide this information.

(3) The purification of the major isozymes of α -galactosidase from human placenta has been accomplished. Major biochemical differences between the two isozymes have been detected including 1) Km values for the artificial substrate 4-methylumbelliferyl- α -D-galactopyranoside, 2) specificity for the natural substrate ceramidetrihexoside and other artificial substrates, 3) susceptibility to neuraminidase and heat treatment and 4) subunit composition.

Then we infused a third Fabry patient with human placental ceramidetrihexosidase in order to examine the effect of this enzyme on the pathological accumulation of ceramidetrihexoside in the patient's kidneys. A satisfactory preinfusion percutaneous renal biopsy was carried out, but the biopsy obtained after administration of the enzyme was not a representative sample of kidney cortex and medulla. Therefore, a comparison of ceramidetrihexoside in these specimens was impossible. Future investigations of enzyme replacement in Fabry's disease will be carried out with open kidney biopsies to insure uniformity and comparability of the samples.

(4) We have demonstrated that administration of hexosaminidase A to a patient with Tay-Sachs disease resulted in about 4 times more total enzymatic activity in the patient's liver than was actually infused. We had previously observed a similar augmentation of enzymatic activity in the liver of a patient with Fabry's disease who received ceramidetrihexosidase. Together these experiments strongly suggest that a subunit of the active exogenous enzyme has caused a configurational change in the mutated catalytically inactive enzymes in the tissues of these patients. If these observations can be substantiated, it suggests that such activation is an important bonus and provides further impetus for enzyme replacement trials in human genetic diseases.

(5) We continue to serve as a center for the diagnosis of patients and detection of carriers for all of the lipid storage diseases. Requests and samples come from all over the world for these assays. Much of our efforts are devoted to the monitoring of pregnancies at risk for heritable metabolic disorders. During the past year we performed more than 297 diagnostic assays for physicians and genetic counselors.

Significance: Enzyme replacement appears to offer considerable promise for the treatment of Gaucher's disease and Fabry's disease. It is expected that the deleterious clinical course in these patients will be ameliorated by this therapy.

Proposed Course: We will continue to carry out and monitor the long-term effects of enzyme infusion in patients with Gaucher's disease and Fabry's disease. The effect of infusing substantially larger doses of enzyme will be investigated. Preliminary studies of enzyme replacement with purified sphingomyelinase will be initiated in patients with Niemann-Pick disease. We shall attempt to design a system to determine whether hexosaminidase A obtained from various sources can be taken up by brain cells. This information is critical for developing a strategy for replacement therapy for Tay-Sachs disease.

Publications:

1. Brady, R. O.: Biochemical genetics in neurology. Arch. Neurol. 33: 145-151, 1976.
2. Brady, R. O.: Complex lipids: from laboratory to layman. Trends in Biochemical Sciences 1: 51-53, 1976.
3. Brady, R. O.: Lipidoses. In D. B. Tower and T. N. Chase (Eds.): The Nervous System, Vol. 2, The Clinical Neurosciences. New York, Raven Press, 1975, pp. 219-277.
4. Golde, D. W., Schneider, E. L., Bainton, D. F., Pentchev, P. G., Brady, R. O., Epstein, C. J., and Cline, M. J. Pathogenesis of one variant of sea-blue histiocytosis. Lab. Invest. 33: 371-378, 1975.
5. Pentchev, P. G., Brady, R. O., Gal, A. E., and Hibbert, S. R.: Replacement therapy for inherited enzyme deficiency. Sustained clearance of accumulated glucocerebroside in Gaucher's disease following infusion of purified glucocerebrosidase. J. Mol. Med. 1: 73-78, 1975.
6. Tower, D. B. and Brady, R. O. (Eds.): The Nervous System, Vol. 1, The Basic Neurosciences. New York, Raven Press, 1975, 685 pp.

Patent

Pentchev, P. G. and Brady, R. O.: Isolation of glucocerebrosidase from human placental tissue. United States Patent No. 3,910,822 issued October 7, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01026-14 DMN
PERIOD COVERED: July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Treatment of Epilepsy--Clinical and Biochemical Study		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Anatole S. Dekaban, M.D., Ph.D., Associate Chief, DMNB, NINCDS OTHER: Michael P. Whyte, M.D., Clinical Associate, DMNB, NINCDS Robert M. Eiben, M.D., Acting Associate Chief, DMNB, NINCDS Elyse Lehman, Ph.D., Psychologist (part time) DMNB, NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Clinical Investigations and Therapeutics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 1.4	PROFESSIONAL: 1.3	OTHER: 0.1
SUMMARY OF WORK (200 words or less -- underline keywords) This is a long range project. Epilepsy is a common neurological disorder often resulting in considerable disability over many years of a patients life. The most effective treatment in the majority of patients is the use of various <u>anticonvulsant agents</u> . There is marked variability of the metabolism of the <u>anticonvulsants</u> from patient to patient. A dosage regimen considered therapeutic may result in considerable slowing of mental performance. Our investigative work includes: 1) Clinical determination of the most effective therapeutic regimen, 2) Chemical determination of <u>plasma levels of the anticonvulsants and their metabolites</u> , 3) <u>Turnover studies</u> of these medications and their metabolites utilizing <u>non-radioactive labelled anticonvulsants</u> , 4) Implementation of special testing to determine changes in patients mental performance with variation of their therapeutic regimen.		

Project Description:

Objectives: In previous years we have contributed to the diagnosis, treatment and long-range management of epilepsy in children. Our current studies are centered on the assessment of the metabolism of anticonvulsant drugs in patients whose seizures are particularly difficult to control. The second important aspect in the management of both children and adults with chronic epilepsy is prevention of excessive drowsiness and slowing of mental activity resulting from high doses of anticonvulsant medication. Since no universally applicable tests for subtle variations in the mental performance in our subjects are available, these need to be developed.

Patient Material: 28 inpatients and 24 outpatients.

Methods Employed:

1. Using metabolic profiles for anticonvulsant agents developed by us (Clin. Neurol. and Neurosurg. 3/4: 168-179, 1974), we have continued to explore variations in drug metabolism in patients whose epilepsy is complicated by thyroid disease or abnormal growth and in patients who in addition to anticonvulsants are receiving certain hormones. Preliminary observations indicate acceleration of drug metabolism in hyperthyroidism.
2. Bio-transformation of phenobarbital to p-hydroxyphenobarbital was studied quantitatively by gas-liquid chromatography in 8 epileptic patients, receiving phenobarbital orally and also in 1 normal volunteer. Following intravenous infusion of a single dose, a) phenobarbital as well as conjugated and unconjugated p-hydroxyphenobarbital were present in the urine of each patient, but m-hydroxyphenobarbital was not detected despite high sensitivity of the assay of 0.25 µg/ml, b) incubation of urines of these patients with enzyme β -glucuronidase liberated conjugated p-hydroxyphenobarbital, but the incubation with sulfatase had no effect; therefore p-hydroxyphenobarbital ranged between 24% and 77% (mean 42%), the recovery of the single dose in a normal volunteer over the period of 16 days after injection amounted to 30%. Phenobarbital, p-hydroxyphenobarbital and m-hydroxyphenobarbital, were not detected in the feces of 4 patients. These results suggest that metabolites other than those listed (and as yet unknown) may be implicated in the elimination of phenobarbital in man; however, degradation of phenobarbital by rupture of the phenobarbital ring can not be ruled out.
3. Fifteen epileptic patients had their dose of anticonvulsant drugs changed twice, each time by 30-50 per cent of the initial medication. Before the dose change, the patients were given 6 specially adapted mental performance tests, which were designed to measure vigilance, reaction time and certain aspects of memory. Serum drug levels were also monitored. The main results, include assessment of effects of drugs on mental performance and evaluation of the psychological tests used. a) Vigilance and reaction time tests were the most useful in

evaluation of effects of various doses of the medication; the memory tasks showed similar, but less definite trends; and rote calculation and block design were of no particular value in the study. b) On the tests for vigilance and reaction time, the greatest number of patients performed best on the lowest dose of their medication, the respective percentages being 45.8 and 56. By comparison, fewest patients performed best on their highest dose, the percentage being 16.7 for vigilance and 12.5 for reaction time; while the percentages on medium dose were 37.5 and 31.2 on the respective tests. c) Use of well-standardized, yet simplified mental performance tests in combination with changes in the dosage of medication can help in reaching a compromise between acceptable seizure control and avoidance of excessive slowing of mental activity.

Significance to Bio-Medical Research and the Program of the Institute:

There are approximately two million individuals in this country with epilepsy. The anguish of the affected patients and their families and economic loss are enormous. Currently, the time has come to place treatment of epilepsy on a better scientific basis with chemical control; this should be associated with prevention of unnecessary impairment of mental performance by excessive medication.

Proposed Course of the Project: The modification of treatment of recurrent cerebral seizures and a better control of the type and dosages of the medication used by determination of balance studies of antiepileptic drugs will continue. There is a need for further refinement in the testing format to assess the patients adaptive and motor performance.

Publications:

1. Dekaban, A.S. and Lehman, E.: Effects of different dosages of anticonvulsant drugs on mental performance in patients with chronic epilepsy. Acta Neurol. Scan. 52: 319-330, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER 00107 00 1 1 0000	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01309-11 DMN
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PERIOD COVERED July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Biosynthesis and Function of Glycosphingolipids and Other Glycoconjugates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: P. H. Fishman, Ph.D., Research Biochemist, DMN, IRP, NINCDS
OTHER: R. O. Brady, M.D., Chief, DMNB, IRP, NINCDS
R. O. Duffard, Ph.D. Visiting Associate, DMN, IRP, NINCDS

COOPERATING UNITS (if any)

Lab. of Molecular Biology, NINCDS, Molecular Biol Sect., VCB, NCI, Sect. on Biochem Cell Reg., LBP, NIAMDS, La. of Cell Metabol., NHLI, Dept. of Immunol., WRAIR.

LAB/BRANCH

Enzymology and Genetics

SECTION

Developmental & Metabolic Neurology Branch

INSTITUTE AND LOCATION

NINCDS, IRP, Bethesda, Maryland 20014

TOTAL MANYEARS:

3.1

PROFESSIONAL:

2.1

OTHER:

1.0

SUMMARY OF WORK (200 words or less - underline keywords)

Although gangliosides are ubiquitous components of cell membranes, the function of these glycosphingolipids is obscure. We are developing several systems to investigate their function. (i) HeLa cells undergo a striking morphological differentiation in response to butyrate. This shape change is associated with induction of a specific glycosyltransferase and an increase in ganglioside content but not with alterations in surface glycoproteins. (ii) Ganglioside GM₁ is the presumed surface membrane receptor for cholera Toxin. A line of chemically-transformed mouse fibroblasts can not synthesize GM₁. These GM₁-deficient cultured cells are unresponsive to cholera toxin; but following binding of [³H]-GM₁ from the culture medium, the cells now respond to the toxin as measured by an increase in cyclic AMP. Other gangliosides can not substitute for GM₁ in these cells. (iii). Certain gangliosides inhibit the binding of thyrotropin to thyroid membranes and the subsequent stimulation of adenylate cyclase. These same gangliosides are found in high amounts in thyroid membranes and induce a conformational change in the hormone. Cholera toxin partially competes with thyrotropin binding, undergoes a conformational change in the presence of GM₁ and has a region of amino acid sequence homology with thyrotropin and related glycoprotein hormones. A similar mechanism of action of cholera toxin and glycoprotein hormones is indicated.

Project Description:

Objectives: To investigate the function of membrane glycosphingolipids in the regulation of cell proliferation, cell morphology, hormone action and toxin sensitivity; to explore the regulation of glycosphingolipid biosynthesis during development and differentiation and relate these findings to anabolic heritable disorders; to determine the underlying mechanism of altered glycosphingolipid biosynthesis in neoplastic tissues; these studies are being extended to other membrane glycoconjugates.

Methods: The glycosphingolipid composition of cultured cells and tissues is determined by extractions and purification of this class of lipids followed by separation of individual glycolipids on thin-layer chromatograms. Metabolism in cultured cells is determined by adding radiolabelled sugars to the culture medium and isolating the labelled glycosphingolipids. Biosynthesis *in vitro* is analyzed by assaying the activities of the glycosyltransferases involved in glycosphingolipid synthesis. Surface glycoconjugates are labelled by selective oxidation with galactose oxidase or periodate and subsequent reduction with sodium borotritide. Gangliosides radiolabeled in specific portions of the molecule are prepared by specific enzymatic reactions. Thus [^{14}C]-N-acetylneuraminyllactosylceramide ([^{14}C]-G_{M3}) is synthesized from lactosylceramide and CMP-[^{14}C] sialic acid with the specific sialyltransferase activity.

Membrane glycoproteins of cultured cells are analyzed by adding ^{14}C -or ^3H -labeled precursors (L-fucose or D-glucosamine) to the culture medium. Surface labeled glycoproteins are removed from intact cells by selective digestion with trypsin and converted to glycopeptides by protease digestion. Glycopeptides are separated by gel filtration column chromatography; glycopeptides from different cell lines such as normal (^3H -labeled) and transformed (^{14}C labelled) are compared by co-chromatography and double-labelled isotope counting techniques.

Major Findings:

A. Studies on Role of Glycosphingolipids in Morphological Differentiation of Cultured Cells. These studies are in collaboration with the Laboratory of Molecular Biology, IRP, NINCDS (Z01-NS-02224-01-LMB). HeLa cells extend neurite-like processes when cultured in the presence of certain short chain fatty acids such as butyrate. We have previously shown that butyrate-treated HeLa have increased amounts of the ganglioside G_{M3} and activity of CMP-sialic acid: lactosylceramide sialyltransferase (~20-fold). The increase in sialyltransferase activity precedes the changes in cell shape and both processes require RNA and protein synthesis. We recently observed that when butyrate-treated HeLa cells are transferred to fresh medium containing low levels of cycloheximide (0.5 $\mu\text{g/ml}$), the cells retain their neurite-like processes for up to 72 hours in the absence of butyrate. Cellular content of G_{M3} remain elevated over untreated cells or butyrate-treated cells transferred to control medium

Henneberry and Fishman, manuscript submitted to Exp. Cell Res.). There is an enrichment of sialoglycopeptides derived from the surfaces of HeLa cells treated with butyrate and decanoate. While both of these fatty acids inhibit HeLa cell growth only butyrate induces morphological changes and G_{M1} biosynthesis. (Fishman and Henneberry, manuscript submitted to J. Biol. Chem.). These results further support the specific involvement of gangliosides in morphological differentiation.

B. Interaction of Cholera Toxin with Ganglioside Receptors on Cell Surfaces. These studies are in collaboration with the Laboratory of Cellular Metabolism National Heart and Lung Institute (Z01-HL-00606-04 LCM). Previous studies have implicated the ganglioside G_{M1} as the specific cell surface receptor for the bacterial enterotoxin cholera toxin. We have used a ganglioside-deficient mouse cell line to directly demonstrate the role of G_{M1} as the toxin receptor. NCTC 2071, a line of chemically transformed mouse fibroblasts, can be propagated in chemically defined medium. These cells lack G_{M1} and do not respond to cholera toxin as measured by activation of adenylate cyclase and elevation of intracellular cyclic AMP levels. When [3H]- G_{M1} is added to culture medium, the cells bind the ganglioside and now respond to the toxin (Moss, Fishman, Manganiello, Vaughan and Brady, Proc. Nat. Acad. Sci. 76, in press). Cyclic AMP accumulation was proportional to the number of molecules of G_{M1} bound per cell and amount of cholera toxin added. Inability of the NCTC 2071 cells to synthesize G_{M1} is due to absence of specific glycosyltransferase activities. Although the cells could bind other exogenous gangliosides such as [^{14}C]- G_{M2} , [^{14}C]- G_{M2} and [3H]- G_{D1a} , they remained unresponsive to cholera toxin. These studies clearly indicate that G_{M1} is the natural and specific receptor for cholera toxin and suggest that gangliosides may function as membrane receptors for other biological active peptides.

C. Interaction of Thyrotropin with Gangliosides. These studies are in collaboration with the Section on Biochemistry of Cell Regulation, Laboratory of Biochemical Pharmacology, National Institute of Arthritis, Metabolism and Digestive Diseases (Z01 AM 23960-09-6BP). Thyroid stimulating hormone (TSH) binds to specific receptors on thyroid membranes. Gangliosides are potent inhibitors of TSH binding; G_{D1b} , G_{T1} and G_{M1} are the most effective inhibitors. These same gangliosides complex with the hormone and induce conformational changes in polypeptide structure based on studies in the ultracentrifuge and spectrophotofluorimeter. In addition, these same gangliosides are found in high amounts in bovine thyroid membranes (Mullin, Fishman, Lee, Aloj, et al, Proc. Nat. Acad. Sci. USA 73: 842-846, 1976). We have also discovered that the B-subunit of thyrotropin (which is the binding subunit) has a region of sequence homology with the B-subunit of cholera toxin (which is its binding subunit). In addition, this same region of similar amino acid sequences is found in other glycopeptide hormones such as luteinizing hormone, human chorionic gonadotropin and follicle-stimulating hormone (Ledley, Mullin, Lee, Aloj, Fishman et al, Biochem. Biophys. Res. Commun., in press, 1976). These observations suggest that all of these peptides may have a similar mechanism of action.

The binding subunits first bind to oligosaccharide chains on the receptor which results in a conformational change in the hormone or toxin structure. This change allows the α or A subunit to activate adenylate cyclase. In support of these concepts we have shown that cholera toxin inhibits the binding of TSH to thyroid membranes (Mullin, Aloj, Fishman, et al, Proc. Nat. Acad. Sci. USA, in press, 1976). In addition, there is a cooperative aspect to the interaction of these two peptides with the membrane receptors. At appropriate concentrations of cholera toxin, binding of TSH can either be increased or decreased. In an analogous manner, TSH can increase the binding of cholera toxin to these membranes.

Significance: These studies are providing information on the function of membrane glycosphingolipids and the regulation of their biosynthesis. We have two important model systems for understanding the function of these complex lipids. One is the dramatic association of increased ganglioside synthesis during cellular differentiation. This model may have important implication for the observed changes in ganglioside metabolism during brain development. The second is the role of gangliosides as membrane receptors for toxins and hormones and their possible role as potentiators of toxin and hormone action by inducing conformational changes in these polypeptides.

Proposed Course: The project will be continued with emphasis placed on the functional aspects of membrane glycoconjugates. We have initiated studies on possible abnormal ganglioside metabolism in thyroid diseases and thyroid tumors. We are also screening sera from patients with thyroid diseases for antibodies against gangliosides. Many of these diseases have an immunological aspect and the detection of such antibodies could have important diagnostic as well as therapeutic implications. We are expanding our observations to include possible ganglioside interactions with other glycoprotein hormones such as human chorionic gonadotropin and luteinizing hormone.

We are now studying a recently born sibling to our described patient with an anabolic sphingolipidosis (Fishman et al, Science 187: 68-70, 1975). The sibling appears to have the same metabolic disease and skin fibroblasts from the patient have been established in culture. We plan in applying our knowledge about cholera toxin-ganglioside interactions in human fibroblasts as a diagnostic and research tool to these cells.

Publications:

1. Bailey, J. M., Fishman, P. H., Kusiak, J. W., Mulhern, S. A., and Pentchev, P. G.: Mutarotase (Aldose-1-Epimerase) from Kidney Cortex. Methods Enzymol. 41B: 471-484, 1975.

2. Brady, R. O.: Composition of membranes of cells transformed by tumorigenic DNA and RNA viruses. Amer. J. Clin. Path. 63: 685-694, 1975.
3. Fishman, P. H., Bradley, R. M., and Menneberry, R. C.: Butyrate-Induced Glycolipid Biosynthesis in HeLa Cells: Properties of the Induced Sialyltransferase. Arch. Biochem. Biophys. 172: 618-626, 1976.
4. Fishman, P. H., and Brady, R. O.: Modification of Membrane Glycolipids by Oncogenic Agents. In Perkins, E. G., and Witting, L. A. (Eds.): Lipid Chemistry and Biochemistry. New York, Academic Press, 1975, pp. 105-126.
5. Fishman, P. H., Brady, R. O., and Aaronson, S. A.: A Comparison of Membrane Glycoconjugates from Mouse Cells Transformed by Murine and Primate RNA Sarcoma Viruses. Biochemistry 15: 201-208, 1976.
6. Fishman, P. H., Moss, J., and Vaughan, M.: Uptake and Metabolism of Gangliosides in Transformed Mouse Fibroblasts: Relationship of Ganglioside Structure to Cholera Response. J. Biol. Chem. 251: in press.
7. Fishman, P. H., Pentchev, P. G., and Bailey, J. M.: Mutarotase from Higher Plants. Methods Enzymol. 41B: 484-487, 1975.
8. Macláren, N. K., Max, S. R., Cornblath, M., Brady, R. O., Ozand, P. T., Campbell, J., Rennels, M., Mergner, W. J., and Garcia, J. H.: GM₃ gangliosidosis: A novel human sphingolipodystrophy. Pediatrics 57: 106-110, 1976.
9. Tanaká, J., Garcia, J. H., Max, S. R., Viloria, J. E., Kamijyo, Y., Macláren, N. K., Cornblath, M., and Brady, R. O.: Cerebral sponginess and GM₃ gangliosidosis: ultrastructure and probable pathogenesis. J. Exp. Neuropath. Neurol. XXXIV 249-262, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01457-10 DMN
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (60 characters or less) The Chemical Synthesis of Radioactive Sphingolipids		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: A. E. Gal Sect. Head, Research Chemist, DMN, NINCDS OTHER: F. J. Fash Bio. Lab. Technician, DMN, NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Neurochemical Methodology Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1
SUMMARY OF WORK (200 words or less - underline keywords) <u>Radioactive isotopes</u> were synthesized and used for <u>metabolic studies</u> and as <u>diagnostic tools</u> in <u>sphingolipidoses</u> . The labelings were made by ¹⁴ C and <u>tritium</u> by <u>gas exposure</u> , <u>synthetic</u> and <u>semi-synthetic techniques</u> and a new approach: <u>Functional group exchange</u> .		

Project Description:

Objectives: To prepare sphingolipids labeled with radioactive isotopes. The compounds are used for metabolic studies and as diagnostic tools in investigations related to hereditary lipid storage diseases.

Methods and Major Findings: A multitude of approaches were used in labelling glycolipids such as chemical synthesis, partial synthesis, minor synthetic modifications, functional group exchange and tritium gas exposure. These methods could be classified into two categories: specific and non-specific labelling. The ideal approach is the specific labelling which consist of the tagging of a complex molecule at a pre-determined atom. Total synthesis is the best way to accomplish this but up to now only few sphingolipids have been synthesized. We synthesized sphingosine, psychosine and galactocerebroside specifically labelled by total synthesis. However, our main effort is directed toward methods which would allow specific labelling of atoms yet would not necessitate tedious syntheses. An interesting technique which we developed is called the functional group exchange. A chemical group such as an acetyl or carboxyl is split from a molecule and is replaced with a similar but radioactive one. With this approach we could prepare aminosugars even gangliosides. Using the approach - minor synthetic modification; we prepared asialo ganglioside, Tay Sachs ganglioside and ceramidetrihexoside. In this approach oxidation and reduction of an alcohol group in the molecule with a radioactive reducing agent would reestablish the original lipid in radioactive form. The lipids used as starting material for this approach were isolated from human tissues. Tritium gas exposure a non-specific approach, was repeatedly used for labelling ceramide dihexoside, dihexoside and globoside. By this method all the non-labile hydrogen atoms in a molecule become radioactive. This procedure is relatively simple but the purification of the resulting compounds are complex. Also this type of compound require more elaborate enzyme assays.

Significance: The compounds are indispensable in the detection, identification and isolation of enzymes connected to lipid storage diseases. Also studies related to qualitative and quantitative determination of enzymes in animal or human tissues necessitate these labelled substrates. Prenatal diagnoses are of rising importance. These labelled compounds play a key role in these diagnostic procedures. As a therapeutic approach, this branch initiated replacement therapy by the administration of the missing enzyme in hereditary diseases. The monitoring of the enzyme levels during and after this therapeutic procedure was done by the use of these radioactive substrates. It would be also of great interest to develop new methods which would allow to prepare relatively easily and inexpensively these compounds for the use of clinicians and for researchers who are not connected to large research centers.

Proposed Course: Work on this project continues in three major directions: 1. Glycolipids will be labeled by using the above mentioned techniques with ^{14}C and Tritium. 2. The approach using "minor synthetic modification" will be extended and used on lipids which were not prepared at all or not prepared by this technique. Also the replacement of the enzymatic oxidation will be explored. 3. Work will continue on the development of the technique: labelling of functional group exchange.

Publications:

See Project Nos. Z01 NS 00815-16 DMN and Z01 NS 00816-16 DMN.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use extra space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01523-09 DMN
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PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Neurology and Biochemistry of Mucopolysaccharidosis; Pathogenesis and
Therapeutic Trials.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Anatole S. Dekaban, M.D., Ph.D., Associate Chief, DMN, NINCDS
George Constantopoulos, Ph.D., Biochemist, DMN, NINCDS

OTHERS: Norio Sakuragawa, M.D., Visiting Fellow, DMN, NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Clinical Investigations and Therapeutics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

See Below

PROFESSIONAL:

OTHER:

SUMMARY OF WORK (200 words or less - underline keywords)

This project was incorporated during 1976 with Project No. Z01 NS 00706-17 DMN.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01808-07 DMN																		
PERIOD COVERED July 1, 1975 through June 30, 1976																				
TITLE OF PROJECT (80 characters or less) Glycoproteins of Myelin in Development and Disease																				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																				
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%; vertical-align: top;">PI:</td> <td style="width: 50%;">R. H. Quarles, Research Chemist</td> <td style="width: 40%;">DMNB, NINCDS</td> </tr> <tr> <td style="vertical-align: top;">OTHER:</td> <td>R. O. Brady, Chief, DMNB</td> <td>DMNB, NINCDS</td> </tr> <tr> <td></td> <td>J. Poduslo, Guest Worker</td> <td>DMNB, NINCDS</td> </tr> <tr> <td></td> <td>L. J. McIntyre, Visiting Fellow</td> <td>DMNB, NINCDS</td> </tr> <tr> <td></td> <td>R. J. McIntyre, Visiting Fellow</td> <td>DMNB, NINCDS</td> </tr> <tr> <td></td> <td>N. Sakurgawa, Visiting Fellow</td> <td>DMNB, NINCDS</td> </tr> </table>			PI:	R. H. Quarles, Research Chemist	DMNB, NINCDS	OTHER:	R. O. Brady, Chief, DMNB	DMNB, NINCDS		J. Poduslo, Guest Worker	DMNB, NINCDS		L. J. McIntyre, Visiting Fellow	DMNB, NINCDS		R. J. McIntyre, Visiting Fellow	DMNB, NINCDS		N. Sakurgawa, Visiting Fellow	DMNB, NINCDS
PI:	R. H. Quarles, Research Chemist	DMNB, NINCDS																		
OTHER:	R. O. Brady, Chief, DMNB	DMNB, NINCDS																		
	J. Poduslo, Guest Worker	DMNB, NINCDS																		
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	R. J. McIntyre, Visiting Fellow	DMNB, NINCDS																		
	N. Sakurgawa, Visiting Fellow	DMNB, NINCDS																		
COOPERATING UNITS (if any) Section on Cellular Neuropathology, LNNS, NINCDS, NIH																				
LAB/BRANCH DMNB Developmental & Metabolic Neurology Branch, LNNS, NIH																				
SECTION Enzymology and Genetics																				
INSTITUTE AND LOCATION NINCDS, Bldg. 10, 3D/17, NIH, Bethesda, Md. 20014																				
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MANYEARS:</td> <td style="width: 33%;">PROFESSIONAL:</td> <td style="width: 34%;">OTHER:</td> </tr> <tr> <td style="text-align: center;">3.3</td> <td style="text-align: center;">2.8</td> <td style="text-align: center;">0.5</td> </tr> </table>			TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	3.3	2.8	0.5												
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																		
3.3	2.8	0.5																		
SUMMARY OF WORK (200 words or less -- underline keywords) The major protein in <u>myelin</u> of the peripheral nervous system is a <u>glycoprotein</u> . Myelin purified from the central nervous system also contains a glycoprotein, but it is quantitatively a minor component of the isolated myelin. This CNS glycoprotein appears to be selectively concentrated in membranes which are transitional between compact myelin and the <u>oligodendroglial surface membrane</u> . We are purifying this glycoprotein which is in the <u>myelin-oligodendroglial complex</u> so that its <u>chemistry</u> and <u>immunogenicity</u> can be studied. This glycoprotein undergoes a chemical change in developing rat brain which may involve its carbohydrate moieties. Since glycoproteins are known to be involved in recognition and contact phenomena, we are investigating its role in <u>developing brain</u> as the oligodendroglial surface membrane makes contact with axons and is spiraled and compacted to form mature myelin. Glycoproteins are also known to be cell surface antigens and receptors for viruses. Therefore, we are investigating the possible role of the myelin-associated glycoprotein in the autoimmune or infectious aspects of <u>multiple sclerosis</u> and other <u>demyelinating diseases</u> .																				

Project Description:

Objectives: To investigate the biochemistry of cells of the nervous system with particular regard to glycoprotein components and their roles in myelination and demyelination. Other myelin and oligodendroglial proteins and lipids will also be examined with the ultimate objective of understanding the molecular mechanisms of myelin formation and breakdown. Emphasis will be placed on the major myelin associated glycoprotein of the CNS and its role in demyelinating diseases such as multiple sclerosis.

Methods: Specific radioactive sugar precursors are used to label CNS and PNS Glycoproteins. Myelin and other subcellular fractions are purified by differential centrifugation on sucrose gradients. Purified myelin is subfractionated into light, intermediate, and heavy fractions with different biochemical and morphological properties. Density gradient centrifugation is also used to isolate other oligodendroglial derived membranes (ODM). Enzyme markers are used to characterize the different subcellular fractions. The membrane-bound proteins and glycoproteins are fractionated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Double label counting techniques are used for detecting the labelled glycoproteins on gels and revealing small differences between samples. Densitometric scanning of gels stained with Fast Green for proteins or periodic acid-Schiff reagent for glycoproteins is used for quantitation of individual protein components. Quantitation of individual lipids is carried out by thin-layer chromatographic separation and densitometric scanning of the TLC plates. Purification of the major myelin-associated glycoprotein involves solvent fractionation, preparative polyacrylamide gel electrophoresis, and column chromatographic techniques.

Glycopeptides are prepared from delipidated myelin by exhaustive digestion with pronase. The soluble glycopeptides are then purified by gel filtration on Bio Gel P-10. Gas liquid chromatography and colorimetric procedures are used for quantitation of individual sugars in glycopeptides and glycoproteins. Labeling of surface glycoproteins of the intact rat spinal cord is accomplished with galactose oxidase treatment and [^3H] sodium borohydride reduction.

Major Findings: The carbohydrate analyses of glycopeptides prepared from central and peripheral myelin have been completed. The glycopeptides were prepared by exhaustive pronase digestion of [^3H] fucose and [^{14}C] glucosamine labeled myelin and purified by gel filtration on Bio Gel P-10. The glycopeptides of PNS myelin eluted as a single sharp peak in which the ^3H and ^{14}C coincided, suggesting a homogeneous fraction. The glycopeptides of CNS myelin eluted as two broad peaks with a varying $^3\text{H}/^{14}\text{C}$ ratio suggesting a substantial amount of heterogeneity. The total glycoprotein-carbohydrate in PNS myelin is about 5-fold higher than that in CNS myelin, which is consistent with our previous finding that the

major protein of PNS myelin is a glycoprotein whereas the glycoproteins associated with CNS myelin are quantitatively minor components. The glycopeptides from both types of myelin contain fucose, mannose, galactose, N-acetylglucosamine, sialic acid, and sulfate. Most of the galactose, sialic acid and sulfate of CNS myelin are in the larger glycopeptide fraction, whereas the smaller fraction, whereas the smaller fraction contains primarily mannose and N-acetylglucosamine.

Substantial progress has been made toward developing a practical procedure for purifying moderate amounts of the most prominent glycoprotein of CNS myelin for chemical and immunological characterization. As reported last year, the binding of this glycoprotein to Con A-Sepharose is complex, possibly due to microheterogeneity in the oligosaccharide moieties of the protein. However, we have now developed conditions for using Con-A-Sepharose whereby a population of myelin glycoproteins which is enriched in the major component can be separated from nonglycosylated proteins and some of the other glycoproteins. This procedure together with solvent fractionation and preparative SDS gel electrophoresis results in about a 75-fold purification of the glycoprotein over myelin. Analytical SDS gel electrophoresis of the fraction shows the major glycoprotein band and 2-3 minor bands which account for only 10-15% of the protein.

As previously reported, double labeling experiments in rats showed that the major fucose-labeled glycoprotein of immature CNS myelin has a higher apparent molecular weight on SDS gels than that in mature myelin. This predominant doubly labeled glycoprotein component from immature and mature rats was partially purified by preparative gel electrophoresis and converted to glycopeptides by exhaustive pronase digestion. Gel filtration on Sephadex G-50 separated the radioactive glycopeptides into several classes which were designated A,B,C and D from high to low molecular weight. The glycopeptides from immature myelin were enriched in the highest molecular weight class relative to those from mature myelin. Neuraminidase treatment of the glycoprotein prior to pronase digestion largely eliminated the developmental differences which were apparent by gel filtration of glycopeptides. This indicates that there is a slightly higher content of large molecular weight, sialic acid-rich oligosaccharide units in the glycoprotein of immature myelin. However, neuraminidase treatment did not reduce the magnitude of the developmental difference revealed by electrophoresing the intact glycoprotein on SDS gels, although it did decrease the apparent molecular weight of the glycoprotein of both immature and mature myelin by an amount comparable to the developmental difference. Therefore, the higher apparent molecular weight for the glycoprotein from immature myelin on SDS gels is not due primarily to its higher sialic acid content. As another approach to elucidating the chemical basis of this developmental change, we are now beginning to characterize trypsin fragments of the glycoprotein from mature and immature myelin. Preliminary experiments indicate that the glycoprotein is very susceptible to mild trypsin treatment of intact myelin and is degraded to at least two fragments with molecular weights of approximately 80,000 and 20,000 daltons, respectively.

Surface labeling experiments on the intact spinal cord with sodium [^3H] borohydride and galactose oxidase have been completed and indicate that most prominent high molecular weight glycoprotein in myelin is at least partially localized on the external surface of the myelin-oligodendroglial complex. The studies also showed that isolated myelin contains a relatively low molecular weight glycoprotein running in the proteolipid region of SDS gels which was very susceptible to surface labeling. This glycoprotein was also labeled in vivo by radioactive-fucose, particularly in long term incorporation studies. Since other laboratories have reported data suggesting that proteolipid is a glycoprotein, we carefully examined this small molecular weight glycoprotein. The results clearly showed that this fucose labeled glycoprotein could be separated from proteolipid on appropriate gel systems and on the basis of solubility in organic solvents. Also we partially purified proteolipid from rat brain and were unable to detect sugars characteristic of glycoproteins by gas liquid chromatography. The results indicate that proteolipid is not glycosylated in rat brain.

Previous studies on CNS myelin subfractions indicated that the major glycoprotein, 2'3'-cyclic nucleotide 3'-phosphohydrolase (CNP), and some of the other high molecular weight proteins are most concentrated in the heavy subfraction. Electron microscopy of this fraction showed many single membranes and vesicles without the classical periodicity of myelin. Our interpretation of these findings is that the heavy myelin subfraction is enriched in membranes which are derived from the oligoplasma membrane but are not compacted into multilamellar myelin. These oligodendroglial derived membranes (henceforth referred to as ODM) may include loose, uncompact myelin, membranes from the inner or outer surface of the myelin sheath which peeled off during isolation, membranes of the processes leading from the oligodendroglial cell body to the myelin, and paranodal membranes which form the junction with the axolemma. Many of these ODM are not recovered in conventional myelin preparations. We have now succeeded in utilizing differential and density gradient centrifugation to isolate membrane fractions which are ordinarily discarded during myelin purification and which have CNP specific activities two to four fold higher than those in conventional myelin. These membrane fractions are also enriched in glycoprotein and other high molecular weight proteins, but contain low levels of basic protein and proteolipid. Electron microscopy shows that these fractions contain little recognizable myelin and are enriched in single membranes. Our interpretation of these results is that these fractions are even more enriched than the heavy myelin subfraction in ODM which are transitional between myelin and the oligodendroglial surface membrane.

Henry Webster and colleagues in LNNS have shown that the optic nerve of *Xenopus* tadpoles provides a useful mode for studying demyelinating agents. The suitability of this model from demyelinating agents such as serum or cerebrospinal fluid of multiple sclerosis patients may depend in part on the biochemical similarity of *Xenopus* myelin to mammalian myelin. We have begun a biochemical analysis of myelin from *Xenopus* brain and spinal

cord. Preliminary results indicate that *Xenopus* myelin contains the major myelin proteins, basic protein and proteolipid, although the basic protein is of slightly higher molecular weight than that in human myelin.

Significance: The probable localization of the major myelin-associated glycoprotein of the CNS in uncompacted myelin membranes and in other oligodendroglial derived surface membranes, as suggested by the sub-fractionation and surface probe studies, has important implications for processes of myelination and demyelination. In this localization the glycoprotein would be accessible for interactions with other cells and with pathological agents such as antibodies and viruses. With regard to myelin formation, there is considerable evidence in the literature indicating that cell surface glycoproteins are involved in recognition phenomena and in specific interactions between cells. The myelin-associated glycoproteins of the CNS could be involved in interactions between oligodendroglial and axonal membranes or between different layers of myelin as they are spiraled and compacted. Many demyelinating diseases such as multiple sclerosis are believed to involve autoimmune and/or viral processes. Membrane glycoproteins are known to be cell surface antigens and receptors for viruses. Therefore, it is reasonable to suppose that the myelin-associated glycoprotein could be directly involved in demyelinating diseases. For example, in multiple sclerosis a viral induced change in the sugars on the glycoprotein could cause it to be recognized as a foreign antigen and subject to autoimmune attack.

For these reasons, information about the chemistry, immunogenicity, and localization of myelin-associated glycoproteins will increase our understanding of the molecular mechanisms of myelinogenesis and demyelination. The carbohydrate analyses of normal adult rat myelin give us a baseline from which to evaluate developmental and pathological changes in these critical membranes which appear to be transitional between the oligodendrocyte and compact myelin. However, the most significant advance is the progress in devising a practical procedure for purifying sufficient amounts of the most prominent myelin-associated glycoprotein for chemical and immunological analysis. The important studies which will be possible with the purified glycoprotein are indicated in the next section.

Proposed Course: One or more additional purification steps will be developed to remove the minor contaminants from the relatively pure glycoprotein preparation which we can now obtain. The amino acid and carbohydrate composition of the purified glycoprotein will be determined. Antisera to the pure glycoprotein will be prepared and immunohistochemical techniques will be used to precisely localize it in the myelin and oligodendrocytes. The purified glycoprotein will be used to determine if multiple sclerosis patients have antibodies or sensitized cells directed against it. Also the fragments of the glycoprotein obtained by mild trypsin treatment of intact myelin will be characterized with particular emphasis on elucidating the chemical reason for the developmental change in the glycoprotein.

A partially purified myelin glycoprotein fraction will be prepared by affinity chromatography on Con A-Sepharose but without the subsequent steps used to completely purify major component. This fraction is enriched in the most prominent myelin glycoprotein and does not contain the major myelin proteins, proteolipid and basic protein. It has the advantage that it can be obtained relatively rapidly in good yield. It will be used to immunize rabbits whose serum will be tested for demyelinating activity on cultured brain explants in collaboration with Dr. Frederick Seil of Stanford University. These experiments are designed to determine if the demyelinating factor in the serum of multiple sclerosis patients or animals with experimental demyelinating diseases such as EAE (especially the chronic forms which more closely resemble multiple sclerosis). The protein, glycoprotein and lipid composition of myelin and ODM fractions will be determined in an attempt to identify the earliest and most significant changes in myelin and oligodendroglial membranes. Also, the protein, glycoprotein and lipid analysis of *Xenopus* myelin will be completed in order to obtain more information about the suitability of *Xenopus* tadpole optic nerve as a model for mammalian demyelinating diseases.

Publications:

1. Matthieu, J.-M., Everly, J. L., Brady, R. O. and Quarles, R. H.: [³⁵S]Sulfate incorporation into myelin glycoproteins. II. Peripheral nervous tissue. Biochem. Biophys. Acta. 392: 159-166, 1975.
2. Matthieu, J.-M., Quarles, R. H., Poduslo, J. F. and Brady, R. O.: [³⁵S]Sulfate incorporation into myelin glycoproteins: I. Central nervous tissue. Biochem. Biophys. Acta. 392: 167-174, 1975.
3. Poduslo, J., Quarles, R. H. and Brady, R. O.: External labeling of galactose in surface membrane glycoproteins of the intact myelin sheath. J. Biol. Chem. 251: 153-158, 1976.
4. Quarles, R. H.: Glycoproteins in the nervous system. In Brady, R. O. (Ed.): NINCDS 25th Anniversary Volume, Basic Neurosciences Section. New York, Raven Press, pp. 493-501, 1975.
5. Quarles, R. H.: Effects of pronase and neuraminidase treatment on a myelin-associated glycoprotein in developing brain. Biochem. J. 156: 143-150, 1976.
6. Zimmerman, A. W., Matthieu, J.-M., Quarles, R. H., Brady, R. O. and Hsu, J. M.: Hypomyelination in copper deficient rats: Effects of prenatal and postnatal copper replacement. Arch. Neurology 33: 111-119, 1976.
7. Zimmerman, A.W., Quarles, R.H., Webster, H.deF., Matthieu, J. M., and Brady, R. O.: Characterization and protein analysis of myelin subfractions in rat brain: developmental and regional comparisons. J. Neurochem. 25: 749-757, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02024-04 DMN
PERIOD COVERED: July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Cerebral Strokes in Patients with Primary Hyperlipoproteinemia.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Anatole S. Dekaban, M.D., Ph.D., Associate Chief, DMNB, NINCDS OTHER: Jan K. Steusing, Research Associate, DMNB, NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Clinical Investigations and Therapeutics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: <div style="text-align: center;">0.6</div>	PROFESSIONAL: <div style="text-align: center;">0.2</div>	OTHER: <div style="text-align: center;">0.4</div>
SUMMARY OF WORK (200 words or less - underline keywords) <p> <u>Cerebrovascular disease</u> is one of the missions of this Institute and we have initiated a study of the role of excessively elevated <u>plasma lipids</u> on the <u>pathogenesis</u> of strokes. The initial phases of this investigation include: 1) The incidence of strokes in patients with <u>primary hyperlipoproteinemia</u>, 2) Assessment of relative frequencies of strokes in various types of hyperlipoproteinemia, 3) The composition of <u>plasma phospholipids</u> in various types of primary hyperlipoproteinemia. The specific laboratory methods include: determination of partitioned plasma lipids and lipoproteins in fasting state. </p>		

Project Description:

Objectives: Cerebrovascular disease is one of the four most common causes of neurological morbidity and mortality. Of various factors predisposing to atherosclerosis is persistent elevation of plasma lipids (whether exogenous - dietary, or endogenous - hereditary hyperlipidemia) and abnormal patterns of lipoproteins. It is our intention to study the content and composition of partitioned lipids and lipoproteins in patients with transient or definitive strokes and in persons at high risk such as members of families with hyperlipidemia. Eventually special dietary measures will be made available to these patients and arrangements will be made for their follow-up.

Material: 171 patients with transient cerebral ischemic attacks or definitive strokes were examined in ambulatory setting and blood drawn following 12-14 hours fasting.

Methods Employed:

1. Detailed personal and family medical history including dietary habits.
2. General medical and neurological examinations.
3. Special examination of the retinal blood vessels and peripheral arteries.
4. Radiological, electroencephalographic, electrocardiographic, and usual routine laboratory surveys of blood and urine.
5. When medically indicated, cerebral angiography and brain scan are performed.
6. Determination of plasma partitioned lipids using established chemical procedures as employed by us in studies of high fat diet in children administered for treatment of epilepsy.
7. Determination of plasma lipoproteins by paper electrophoresis, and when indicated by polyacrylamide gel electrophoresis and density gradient centrifugation.

Major Findings: Out of 171 patients with strokes, 22 proved to have primary hyperlipoproteinemia by paper electrophoresis and by elevation of cholesterol over 300 mg/100 ml or triglycerides over 200 mg/100 ml or both these components. Of the 22 patients, more than two-thirds had at least one close relative with elevated blood lipids. Using criteria of the World Health Organization, these patients were classified as follows: 5 had hyperlipoproteinemia type IIa, 8 had type IIb, 3 had type III and 6 had type IV. Phospholipid content and composition showed relatively little difference from normal control values. The numerical distribution of patients with stroke and hyperlipoproteinemia into the four different types corresponded quite well with the approximate frequency of these types in the general population. Thus, this study does not indicate that the patients with a particular type of hyperlipoproteinemia are at a greater risk to have a stroke than those belonging to other types.

Significance to Bio-Medical Research and the Program of the Institute:

One of the title words of this Institute is "Stroke" and there is clearly defined obligation to investigate the etiology, pathogenesis and preventive measures of cerebrovascular disease. Primary hyperlipoproteinemia is associated with permanent elevation of principal plasma lipids: cholesterol or triglycerides or both. For this reason young patients with familial hyperlipoproteinemia are particularly suited for studies on cerebrovascular disease.

Proposed Course of the Project: This project was just begun; because of its promise and valuable initial results, it will be continued during years to come. First paper has been submitted for publication.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-NS-02103-04 DMN

PERIOD COVERED

July 1, 1975 - June 30, 1976

TITLE OF PROJECT (80 characters or less)

Investigations of Pathogenesis and Assessment of Therapeutic Trials in
Fabry's Disease and Gaucher's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. S. Dekaban, Assoc. Chief, DMN, NINCDS
OTHER: R. O. Brady, Chief, DMN, NINCDS
P. G. Pentchev, Biochemist, DMN, NINCDS
A. E. Gal, Organic Chemist, DMN, NINCDS
M. Zelkowitz, Clinical Assoc., DMN, NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Clinical Investigations and Therapeutics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

1.0

PROFESSIONAL:

0.5

OTHER:

0.5

SUMMARY OF WORK (200 words or less - underline keywords)

Enzyme replacement trials were carried out in two patients with Fabry's disease and three patients with Gaucher's disease. The quantity of accumulating lipid in the blood and liver decreased following injection of the requisite enzymes.

Project Description:

Objectives: The underlying abnormality in Fabry's disease is deficiency of an enzyme, α -galactosyl hydrolase, which is necessary for catabolism of a glycosphingolipid: galactosylgalactosylglucosylceramide. The disorder is transmitted as an X-linked trait and is characterized clinically by telangiectatic skin lesions (angiokeratoma corporis diffusum), corneal opacities, intractable burning pain in the extremities, progressive renal dysfunction and general fatigue. The life span is shortened, death occurring in early to mid-adult years usually from renal failure, cardiac, or cerebral complications.

The basic abnormality in Gaucher's disease is a deficient enzyme activity of β -glucosidase, which catalyzes removal of glucose from glucosylceramide. This results in excessive accumulation of this glycolipid in the cells of reticulo-endothelial system, leading to life threatening manifestations--hepatosplenomegaly, skeletal abnormality and neurological involvement.

We have demonstrated (New Engl. J. Med. 289:9-14, 1973) that intravenous infusion of large volume of completely matched white blood cells or of purified enzyme--ceramide trihexosidase to the patients with Fabry's disease can produce transient improvement in biochemical abnormality in the form of a substantial decrease of excessively accumulating glycolipid. Similar results were obtained after infusions of glucocerebrosidase to two patients with Gaucher's disease (New Engl. J. Med. 291: 989-993, 1974).

In order to produce amelioration of the clinical abnormality, a repetitive long-term supply of the deficient enzymes to these patients would have to be provided. Organ transplantation (kidneys), successive infusion of blood products or of purified enzymes are very involved and expensive procedures and are still in an experimental phase. Proper selection of patients and establishment of specific indication for such therapeutic trials is now in order and in progress.

Material: 7 inpatients and 25 outpatient visits.

Methods: Active enzymes, glucocerebrosidase and ceramidetrihexosidase are obtained from human placenta by extraction and fractionation with ammonium sulfate. Subsequently the enzyme is purified by passages on Sephadex G-200 columns, ion exchange chromatography including DEAE and Sephadex cation exchange columns. The final enzyme proteins are homogeneous and pure. The enzymes are suspended in albuminated saline and submitted to the NIH Pharmaceutical Development Service for study of sterility and pyrogenicity in order to obtain their clearance for intravenous administration to the patients.

Infusion of the enzymes will be preceded and followed by examination of the blood, liver and kidney tissues obtained by needle or open biopsy. This will permit determination of the quantitative change in the excessively accumulating unmetabolized substrate.

Medical literature from 1920 to 1973 was scrutinized for detailed case reports on Fabry's disease. The data thus obtained plus the data from 7 of our own patients was analyzed for age of onset of the disease, predominant clinical abnormality and complications.

Major Findings:

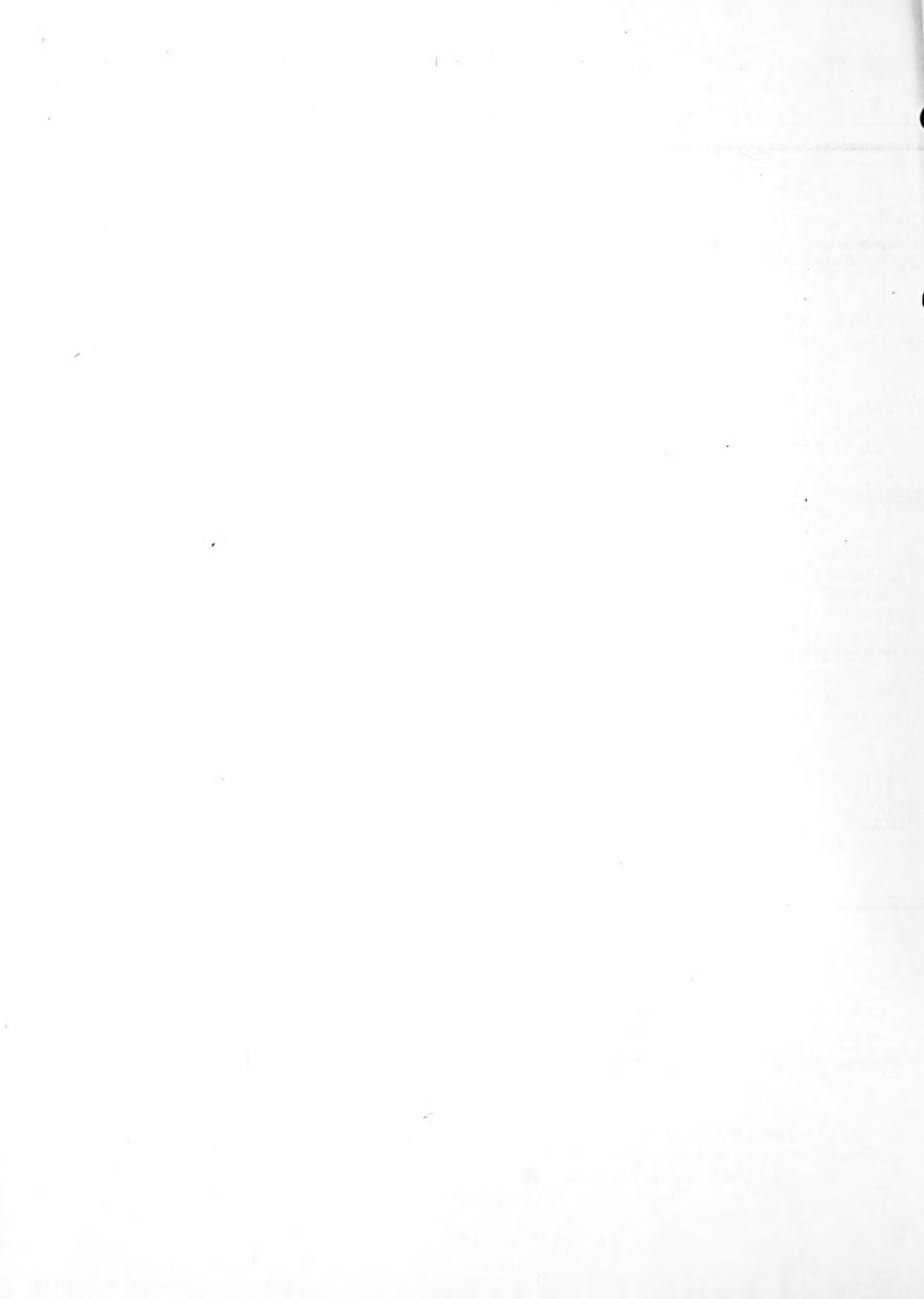
1. Intravenous infusions of purified glucocerebrosidase to two patients with Gaucher's disease caused a substantial decrease in the quantity of glucocerebrosidase in erythrocytes of these patients. Likewise there was a decrease of this glycolipid in the liver following enzyme infusion as compared to the preinfusion value. There was no obvious change in patient's clinical condition following enzyme infusion and actually this was not expected after only two consecutive infusions. The theoretical value of these trials to correct the biochemical errors are far reaching and further infusions are planned.

2. Clinical data on 80 patients with Fabry's disease were evaluated for age of onset, characteristics of life history of the disease and the complications. This was done in order to provide criteria for better selection of patients for enzyme replacement trials and lay down the indications. The mean age of onset of Fabry's disease in 48 males still alive was 10.2 years and in 17 males who died, the mean age of onset was 8.1 years. This suggests that eventual enzyme replacement therapy to be effective should start early, before irreversible changes occur. The mean survival age of 23 patients who died was 38.7 years. Autopsy examination showed the presence of renal damage in all patients; the coexisting complications, included occlusive condition of coronary arteries, liver involvement and cerebrovascular disease.

Proposed Course of the Project: This project will be terminated as a separate entity and the principal activities will be subsumed under Project No. Z01 NS 00815-16 DMN.

Publications:

1. Dekaban, A. S., and Zelkowitz, M.: Fabry's disease: evaluation of age of onset and natural history as preliminary steps to replacement therapy trials. Clin. Proc. Child. Hosp. Natl. Med. Ctr. XXXI: 223-228, 1975.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02128-02 DMN
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Inborn Error of Copper Metabolism: Kinky Hair Disease. Determination of Defect, Therapy.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Anatole S. Dekaban, M.D., Ph.D., Associate Chief, DMNB, NINCDS OTHER: Jan K. Steusing, Research Associate, DMNB, NINCDS Roger Aamodt, Ph.D., Senior Investigator, NMD, CC Warren Rumble, B.Sc., Research Associate, NMD, CC		
COOPERATING UNITS (if any) Nuclear Medicine Department, Clinical Center, NIH		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Clinical Investigations and Therapeutics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 1.1	PROFESSIONAL: 0.4	OTHER: 0.7
SUMMARY OF WORK (200 words or less - underline keywords) In the course of study of <u>inborn errors of metabolism</u> , we have encountered four patients with <u>Kinky hair disease</u> , a rare sublethal disorder. The condition was presumed to be caused by <u>absorptive defect of copper</u> but the extent of the deficit was not known. Using intravenous and oral administration of <u>labelled ^{67}Cu</u> to the same patients, we demonstrated the extent of absorptive defect of copper to be in the range of 11-13 per cent. These patients retain the absorbed or infused copper 3-4 times longer than unaffected controls. Subsequently, we have modified the method of <u>parenteral administration of copper</u> by using subcutaneous infusion in low concentration to permit long range ambulatory therapy in young infants.		

Project Description:

Objectives: Kinky hair disease (KHD) is a genetically determined disorder of copper metabolism. It is inherited as an X-linked recessive trait and so far the condition has been sublethal. The main clinical features of this disease include epileptic attacks, mental deterioration, spasticity and brittle hair. The last feature resulting from abnormal cross-linking of keratin which is copper dependent. Pathological abnormalities include, cerebral degeneration and defects of elastica interna of arteries. The main laboratory findings include very low plasma copper (less than 12% of normal), low ceruloplasmin (less than 20% normal) and low tissue copper. The underlying abnormality of the disease seems to be related to impaired absorption of copper from the gastrointestinal tract. This required confirmation and determination of the extent of the defect.

This project aims to study absorption and turnover of labelled copper ^{67}Cu in patients with KHD, and normal controls. Knowledge of the percentage of copper absorption by KHD patients will facilitate development of appropriate therapy.

Patient Material: 4 inpatients.

Methods Employed:

1. Determination of serum copper by dilution method utilizing atomic absorption spectrophotometer. Determination of ceruloplasmin using p-phenylenediamine method of Ravin.
2. Intravenous and at later date oral administration of ^{67}Cu (half-life 61.7 hours).
3. Daily measurement of total body ^{67}Cu radioactivity using 60 cm arc with two 8 x 4" NaI (TI) scintillation detectors and Packard Instrument 1024 Channel Pulse Height Analyzer. An injection or ingestion duplicate to be counted just prior to each measurement on the patient, and resulting values to be used to correct patient data for physical decay of ^{67}Cu .
4. Probe measurements to be made using a Nuclear-Chicago dual probe and a Hamner Electronics Co., scaler.
5. Radioactivity of stools, urine and blood to be measured daily.

Major Findings:

Intravenous and oral administration of ^{67}Cu to the same subject permitted us a precise calculation of the percent absorption of labelled ^{67}Cu .

- (1) The measured gastrointestinal absorption of the labelled copper in 3 patients with KHD ranged between 11 and 13% of normal.
- (2) The patients with KHD retained the injected or absorbed labelled copper 3-4 times longer than normal controls. This indicates increased demand for copper and possible recycling of this element in the body.

- (3) The retained ^{67}Cu remained longer in the liver; the uptake of copper by the red blood cells was almost normal, indicating preferential utilization of the plasma copper, even though its level was low.
- (4) Kinky hair disease is a sublethal condition with the onset of symptoms under 3 months of age. After demonstration of the extent of absorptive defect of the copper from gastrointestinal tract we have embarked on therapeutic trials. Conjugation of copper with chelating agents, amino acids or peptides failed to increase absorption of copper. However, we have developed a therapeutic regimen which corrects the biochemical defect indefinitely and maintains the serum copper and ceruloplasmin at normal levels, ameliorating the patient's clinical condition. Essentially the treatment consists of subcutaneous infusion of 1-2 mg of copper sulfate in 25-50 ml of saline (0.04 mg of CuSO_4/ml) given by slow drip over 1-2 hours. The infusions are given every 3-4 days in four alternate sites: two beneath the lower border of the scapula and two at the level of the eighth rib in the midscapular line. We were able to maintain this therapy for seven months with satisfactory results. The reason for this manner of administration is a high toxicity of copper salt to the tissue unless greatly diluted. Intravenous infusions of large volume of saline containing copper salt proved impractical in young infants and could not be maintained on an ambulatory basis. Using the method of subcutaneous infusion of copper sulfate in two patients with KHD we were able to correct the biochemical defect: plasma copper and ceruloplasmin were brought to normal values and maintained at this level for a long time. The clinical course of the patients was improved (Dekaban and Steusing, Lancet, Dec. 21: 1523, 1974).

Significance to Bio-Medical Research and the Program of the Institute:

Demonstration of the greatly decreased but not absolute lack of the absorption of oral copper will have important implications in devising therapy for the patients with KHD. We have already tried to administer orally conjugated copper with various chelating agents and polypeptides, so far this was without success but further approaches are indicated. We were successful in devising the parenteral therapy with copper sulfate in the form of large subcutaneous infusions. This permitted complete correction of the biochemical abnormality in the plasma by reversion of the copper and ceruloplasmin to normal levels.

Proposed Course of the Project: The objectives were reached, results published and this project is considered completed.

Publications:

1. Dekaban, A.S., Aamodt, R., Rumble, W.F., Johnson, G.S. and O'Reilly, S.: Kinky hair disease. Study of copper metabolism utilizing ^{67}Cu ; some reference to Wilson's disease. Arch. Neurol. 32: 672-675, 1975.

2. Dekaban, A.S.: Inborn errors of copper metabolism: Kinky hair disease and hepatolenticular degeneration. Therapeutic approaches. Materia Medica Polona (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02129-02 DMN
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Biochemical Characterization of Mucopolysaccharidosis Type VI (Maroteaux-Lamy).		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: George Constantopoulos, Ph.D., Biochemist, DMNB, NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Clinical Investigations and Therapeutics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: None
SUMMARY OF WORK (200 words or less - underline keywords) Characterization of the <u>urinary glycosaminoglycans</u> (GAG) excreted by a patient with <u>MPS-VI</u> (Maroteaux-Lamy). Determination of the amount, composition and molecular weight distribution of the urinary GAG chemically defines MPS-VI and may help in the diagnosis.		

Project Description:

Objectives: The objective of this study is the characterization of the urinary glycosaminoglycans (GAG) excessively excreted by a patient with mucopolysaccharidosis type IV (MPS-VI) also known as Maroteaux-Lamy disease. This material presumably represents the substrate of the enzyme which is deficient in these patients. The solution of this problem may help in the diagnosis and it may increase our understanding of the absence of cerebral involvement--as compared to the patients with other types of mucopolysaccharidosis.

Material: Urine from a patient with type MPS-VI.

Methods Employed: Isolation and measurement of total GAG from urine; fractionation of the individual GAG by ion exchange chromatography on Dowex 1; further purification of dermatan sulfate and chondroitin sulfates by copper precipitation, and/or depolymerization with chondroitinase AC. Characterization of the disaccharides produced after incubation with chondroitinase AC and of the remaining GAG with chondroitinase ABC. Chemical analysis. Acetate electrophoresis.

Major Findings: 1. Presence of mucopolysacchariduria. 2. The GAG consist of chondroitin-4-sulfate (about 20%) and dermatan sulfate (about 80%). There was not excessive excretion of heparan sulfate. 3. A sizable part of dermatan sulfate was broken down to small fragments. Molecular weight distribution of the urinary GAG was characteristic of this disease.

Significance to Bio-Medical Research and the Program of the Institute:

The understanding of biochemical abnormality in type VI MPS will be considerably aided by characterization of the material which is not metabolized and which affects adversely certain body organs and tissues, but spares the brain. Other forms of MPS are usually associated with severe cerebral abnormalities. The study of genetic neurological disorders is the objective of this section and of the Institute.

Proposed Course of the Project: Recently, the deficient enzyme in MPS-VI, that is sulfatase B, has been identified as N-acetylgalactosamine-4-sulfatase. It is of interest that both dermatan sulfate and chondroitin-4-sulfate, which in our study were found to be excreted excessively in the urine of the patient with MPS-VI, contain N-acetylgalactosamine-4-sulfate.

In the course of our studies we have encountered 4 patients who clinically resembled MPS-V, but chemically belong to MPS-VI. Two of those patients were available and we have now shown that both have Arylsulfatase B deficiency. We intend to fully document these patients.

Publications:

1. Constantopoulos, G. and Dekaban, A.S.: Chemical definition of mucopolysaccharidoses. Clinica Chimica Acta. 59: 321-336, 1975

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (to 100 and 3 spaces)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02162-02 DMN
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) Synthesis of Compounds Analogous to Glycolipids		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: A. E. Gal, Research Chemist, DMN, NINCDS OTHER: F. Fash, Bio. Lab. Technician, DMN, NINCDS		
COOPERATING UNITS (if any) None.		
LAB/BRANCH Developmental & Metabolic Neurology Branch		
SECTION Neurochemical Methodology Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 1.4	PROFESSIONAL: 0.7	OTHER: 0.7
SUMMARY OF WORK (200 words or less - underline keywords) Compounds were synthesized which are <u>glycolipid analogs</u> and which give a <u>chromophoric moiety</u> upon enzymatic hydrolysis. These compounds are used in <u>enzymatic research</u> and as <u>diagnostic reagents</u> in <u>Niemann-Pick</u> , <u>Gaucher's</u> and <u>Krabbe's disease</u> .		

Project Description:

Objectives: The compounds to be synthesized in the framework of this project are molecules similar to glycolipids which when cleaved enzymatically provide a chromophore useful for the diagnosis of lipid storage diseases and for the identification of heterozygous carriers.

Methods and Major Findings: Further work was done related to improvements of the synthesis of 2-hexadecanoylamino-4-nitrophenyl phosphorylcholine (HNP). This substance resembling sphingomyelin but having a benzene ring instead of the aliphatic chain gives due to its nitrophenyl moiety intense coloration upon enzymatic attack. It is a reliable chromogenic substrate used for assaying sphingomyelinase activity in diverse human tissue samples. It is used for the diagnosis of homozygotes and detection of heterozygous carriers of Niemann-Pick disease. Based on the chemistry of this compound, the research on non-radioactive substrates were extended to other lipidoses. Compounds were synthesized which could be used as substrates for measuring gluco and galactocerebroside levels in tissue extracts. 2-Hexadecanoylamino-4-nitrophenyl glucoside showed to be a useful compound for the diagnosis of Gaucher's disease. 2-Hexadecanoylamino-4-nitrophenyl galactoside can be used for the diagnosis of Krabbe's disease. Work is also going on on the synthesis of a substrate which would make feasible the diagnosis with a chromogenic substrate in Farber's disease, a disease characterized by the lack of ceramidase.

Significance: These new compounds were thoroughly tested and they have been found useful for the diagnosis of lipid storage diseases. These findings are a major breakthrough because the radiolabeled products are scarce, expensive, and not widely available. The chromogenic substances can be used and easily handled by practitioners and clinical chemists with no danger of radioactive contamination and they eliminate the necessity of costly and complex radioactive scanning techniques.

Proposed Course: Based on the basic idea established by this project, compounds will be synthesized with chromophoric moieties which hopefully will be specific for the detection of other enzyme deficiency disorders.

Publications:

1. Gal, A. E., Brady, R. O., Hibbert, S. R. and Pentchev, P. G.: A practical chromogenic procedure for the detection of homozygotes and heterozygous carriers of Niemann-Pick disease. New Engl. J. Med. 293: 632-636, 1975.
2. Gal, A. E., and Fash, F. J.: Synthesis of 2-hexadecanoylamino-4-nitrophenyl phosphorylcholine hydroxide, a chromogenic substrate for assaying sphingomyelinase activity. Chem. Phys. Lipids 16: 71-79, 1976.

3. Gal, A. E., Pentchev, P. G. and Fash, F. J.: A novel chromogenic substrate for assaying glucocerebrosidase activity. Manuscript submitted.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (A 20 character space)	U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02163-02 DMN
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PERIOD COVERED: May 1, 1975 through June 30, 1976

PROJECT (80 characters or less)

Development of Special Analytical Methods and Preparative Techniques
to investigate the Etiology and Therapy of the Sphingolipidoses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. E. Gal, Section Head, Research Chemist	DMN	NINCDS
OTHER:	F. J. Fash, Biol Lab. Technician	DMN,	NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Neurochemical Methodology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

New analytical techniques were developed and used in enzymatic research and in clinical investigations of lipidoses. Enzyme purification was studied by affinity chromatography. The lipid content in human tissues, the diagnosis of lipid storage diseases by gas, thin-layer chromatography and other techniques were studied at the microgram level. Also preparative work was done and used in connection with further synthetic work and for the preparation of lipid standards.

Project Description:

Objectives: To develop techniques by which the separation and chemical analysis of biologic materials related to sphingolipidoses can be advanced. This work involves the following approaches: 1. Improvement of techniques leading to the separation of enzymes. 2. Development of ultramicro analytical methods for the determination of lipids in biological materials.

Methods: 1. Work on the separation of enzymes is related to affinity chromatography, a technique whereby an enzyme is temporarily fixed to a column containing a molecule (ligand) which reacts with an enzyme by immobilizing it. An insoluble inert support (such as agarose) is bound to a spacer arm which contains the ligand. The latter is usually a synthetic compound similar in its complexity to the substrate with which the enzyme interacts. A great number of ligands were synthesized by us and the corresponding affinity columns were assembled. 2. The development of methods for the determination of lipids in small samples of biological materials of human origin such as erythrocytes, leukocytes, fibroblast serum, cerebrospinal fluid, urine or biopsy samples from kidney, liver and brain. The individual sphingolipids are present usually only in submicrogram quantities in these samples. For the separation of such lipids, thin layer and gas chromatographic procedures combined with column-liquid chromatography was used.

Quantitative evaluation was made by scanning of the thin-layer plates or by gas chromatography. Much work was done in areas not covered by existing literature references.

Major Findings: Improved purification of the enzymes were achieved by using affinity chromatography systems. Considerably more work has to be done before the advantage of these procedures could be evaluated in gains in man-hour work. Gas chromatography of glucose originating from lipids could not be determined previously. This problem was solved by us. Also a new thin-layer chromatography system was developed which resulted in more reliable results using only small amounts of specimen. A novel technique was developed in which lipids present in the same sample (but not attacked by the exogenous enzyme) were used as internal standards. Improved analytical techniques showed practical results particularly in the studies related to replacement therapy of enzymes where the decrease of lipid levels in the liver and erythrocytes of patients could be established and through these procedures, evaluation of the therapeutic administration of enzymes can be assessed.

Significance: The purification of the missing enzymes required for the therapy of the lipid storage diseases is a complex, tedious, and costly procedure. The use of affinity chromatography should provide a significantly simplified method. The identification of accumulated lipids in human tissues for the diagnosis and control of inherited lipid diseases is dependent on the sensitivity of the analytical techniques. The

importance of accuracy in working with trace amounts of material in biological specimens necessitates improved techniques at the submicrogram level.

Proposed Course: Efforts to improve the purification of enzymes by affinity chromatography or by other chemical operations will continue as well as by utilizing other advanced techniques. Much more work has to be done in relation to the improvement of microanalytical procedures; for example, the ultramicrodetermination of aminosugars and sialic acid needs further development. Some of the existing methods are too complex and their simplification will be investigated. The application of other techniques including high speed (or pressure) liquid chromatography or the use of mass spectroscopy will be explored.

Publications:

See Project No. Z01 NS 00815-16 DMN

ANNUAL REPORT
July 1, 1975 through June 30, 1976
Laboratory of Neuropathology and Neuroanatomical Sciences, IRP
National Institute of Neurological and Communicative
Disorders and Stroke

Igor Klatzo, Chief

The major research efforts of this laboratory have been directed towards further elucidation of pathophysiology of cerebral ischemia, demyelinating diseases, and mechanisms of synaptic transmission.

Cerebral ischemia was studied in the three sections of the LNNS using the Mongolian gerbils as an experimental model.

In the Section on Cerebrovascular Pathology, effects of increased systemic blood pressure (SBP) were evaluated in the postischemic periods. It was demonstrated that elevated SBP following re-establishment of circulation produces a markedly deleterious effect expressed by a more severe histological injury, marked retention of the lactate, and accelerated damage of the blood-brain barrier (BBB) in the hypertensive groups of animals. With additional evidence of increased transport of various substances into the parenchyma in the hypertensive animals, it appears that the main cause for increased brain tissue damage in the hypertensive gerbils is the reduced transport out of the brain parenchyma of various waste and toxic products, such as lactate.

The studies on the behaviour of biogenic amines indicated that ischemia results primarily in an increased release of the amines and reduction of their levels in the brain tissue. On the other hand, there is an accumulation of the metabolites of dopamine, norepinephrine and 5-HT due to their inhibited out-transport from the brain, as it can be deduced from the similarity in the action of probenecid and ischemia on the pargyline treated animals.

Application of graded kerosine-bromobenzene column method of Nelson for measurement of the specific gravity of the brain tissue allowed an estimation of water uptake in even small specimens taken from various anatomical brain structures. Thus it was possible to outline dynamic profiles in the development of ischemic brain edema. The specific gravity measurement revealed that following onset of ischemia there is an appreciable uptake of water within 10 minutes. Three to five hours after re-establishment of the circulation two groups of animals can be recognized: 1) gerbils in which edema persists at the same level and the animals do not show any extensive necrosis or BBB damage, and 2) gerbils which reveal extremely low specific gravity, necrotic foci and damage of the BBB.

Otherwise, increased permeability of the BBB, which introduces an element of vasogenic edema into ischemic injury, can be recognized as being of two types: 1) dependent on the increased vesicular transport across the endothelium and 2) due to mechanical disruption of endothelium in the necrotic foci. The problem of ischemic edema is of considerable clinical importance because a control or reduction of the abnormal water uptake can greatly influence the neuronal recovery and the clinical prognosis. Our current experiments are thus designed to elucidate various mechanisms involved in ischemic edema formation.

The Section on Neurocytobiology continued to investigate the cerebral transport phenomena altered by pathological conditions especially those of ischemia and to characterize the histochemical and biochemical properties related to the transport function of capillaries, glia, and neurons grown in organotypic cerebellar culture of the cultured microvessels in the pia arachnoid explants. The most important observations were as follows: 1) In ischemia, the vesicular transport in cerebral arterioles, venules, and capillaries progressively increased with the duration of cerebral blood supply deprivation which, otherwise, never resulted in degeneration of the endothelial cells in the brain vessels. 2) The progressively decreased specific 2-deoxy-D-glucose uptake in the synaptosomes, seen during cerebral ischemia of 30-180 minutes, returned to the level of controls 1 hour after re-establishment of cerebral circulation. It appears, at least initially, the synaptosomal transport function can be restored following ischemia of 1-3 hour duration. 3) A simple method was developed to isolate and enzymatic and metabolic active fraction of cerebral microvessels from nonvascular brain tissue which showed a saturable uptake of 2-deoxy-D-³H glucose. The K_m for ³H 2DG was .1 mM and the uptake was inhibited by 3-O-methyl-D-glucose, the unmetabolizable glucose analogue and by phlorizin and phloretin the inhibitors of glucose carrier transport. 4) The cerebral capillary fraction obtained for rabbits subjected to oxygen saturation or pCO₂ tension change showed greater reduction of 2DG uptake in hypoxic than hypercapnic and an increased uptake in hypocapnic when the uptakes were calculated as per cent of control uptake. The effects of an altered pO₂ saturation and pCO₂ tension on the capillary fraction were similar to the one obtained in the rabbit brain studied in vivo suggesting that the cerebral glucose uptake may be directly related to the capillary function.

The comparative studies of amino acid uptake in pia arachnoid explants and fetal fibroblastic culture have shown that the uptake of both the metabolizable isoleucine and the unmetabolizable cycloleucine take place by facilitated carrier mechanism. However, the pia arachnoid is distinctly different from the fibroblasts in being unable to accumulate the amino acid. For example, the concentration of isoleucine in the pia arachnoid explants never exceeded the concentration of the amino acid in the medium, while the fibroblastic uptake was 12 times higher than level of isoleucine in the incubating media. The myelinated cerebellar cultures composed of glia, neurons, and granular cells, but almost devoid of capillaries, took up the unmetabolizable 3MG and the partially metabolizable 2DG by NaCl independent facilitated carrier mechanism in 60% and by diffusion in 40% at pH 7.0. The

Km, that is the concentration of half maximal uptake of 3MG and 2DG was 11 μ M respectively. Ultrastructurally, the capillaries grown in organotypic cerebellar cultures showed the same characteristic features as the one in situ. For example, the intercellular tight junctions were identifiable and the alkaline phosphatase was confined to the plasma membrane. Two types of ATPase activity were demonstrated in the cerebellar cultures. In the glia and neuropil, the enzyme activity was associated with $(\text{Na}^+ - \text{K}^+)$ active transport system since the enzymatic reaction was inhibited by 1 mM ouabain or 1 mM showdomyacin. In the neurons, the ATPase reactivity was enhanced by ouabain and its function remains to be clarified.

In the Section on Cellular Neurochemistry, a major project has been concerned with the study of biochemical changes during ischemia and recovery following ischemia. Unilateral ischemia in Mongolian gerbils was accomplished by ligation of the left common carotid artery. The effects of ischemia after varying periods of ligation, the recovery after recirculation, and the susceptibility of the brain to a second period of ischemia were examined. The energy reserves of brain, P-creatine, ATP, glucose and glycogen were measured, as well as lactate, glutamate, γ -amino butyric acid, cyclic AMP, cyclic GMP, dopamine, norepinephrine and 5-hydroxytryptamine. It was found that the brain was hyper-reactive to a second ischemic insult after one hour of recirculation, and was hyporeactive following 5 and 20 hours of recirculation. The significance of the altered response to a second ischemic episode is presently unclear, but may be important to the clinical situation where multiple insults are not uncommon. A similar phenomenon has also been observed histologically in the hippocampus.

Additional studies on short-term effects of bilateral ischemia have been performed. The cyclic AMP levels showed an initial (1 min. of ischemia) large 18-fold increase followed by a decrease to levels only 5-fold greater than control at 5 min. of ischemia. After unilateral ischemia, there was a large post-ischemic rise in cyclic AMP levels at 5 min. of recirculation after 5 min. of ischemia, in contrast, there was no postischemic increase at 5 min. after 1 min. of bilateral ischemia. Pretreatment of the gerbils with 100 mg/kg of theophylline prevented the ischemic-induced increase in cyclic AMP, but not the postischemic-induced increase. Whether the effect of theophylline is related to its ability to improve survival following an ischemic episode or not is currently under investigation.

The effect of oxygen and glucose deprivation in C-6 astrocytoma and C-1300 neuroblastoma cells in culture is being investigated as a corollary to the ischemic studies in gerbils.

Another project has been concerned with the effects of seizures induced either by electroshock or drugs (isoniazid and pentylenetetrazole) on γ -amino butyric acid (GABA) and cyclic GMP in the cerebral cortex and cerebellum of mouse brain. One group of anticonvulsant drugs, such as dipropylacetic acid, increased GABA and decreased cyclic GMP in the cerebellum. Another group of drugs, including phenobarbital and phenytoin decreased the cyclic GMP but had no effect on the GABA in the cerebellum. Neither group had a significant

effect on the cyclic GMP in the cerebral cortex. Those agents which affected the GABA levels had similar effects in both the cerebral cortex and cerebellum. During the period of increased GABA and decreased cyclic GMP concentrations, the threshold for seizure as determined by electroconvulsive shock was increased.

Convulsants such as isoniazid and pentylenetetrazol increased the cyclic GMP in both cerebellum and cerebral cortex. None of the pharmacological manipulations tested significantly affected the levels of cyclic AMP. Maximal electroshock increase both cyclic AMP and cyclic GMP in the cerebral cortex and cerebellum. Pretreatment of the mice with 20 mg/kg phenytoin, a dose which diminishes the convulsive response, reduced the cerebellar cyclic GMP response by 50 per cent but had little or no effect on the other cyclic nucleotide changes.

The GABA shunt metabolites and enzymes are also being examined in C-6 astrocytoma and C-13000 neuroblastoma cells in culture. GABA in neuroblastoma cells is found to be one-tenth the concentration found in glioma cells, while GABA transaminase activity is either lacking in astrocytoma cells, or is less than one-tenth of the activity in neuroblastoma cells. The cells afford an opportunity to compare the effects of anticonvulsants and inhibitors of the GABA pathway on cells of glial and neural origin.

The regulation of glycogen metabolism in cell cultures of neuronal origin has been examined. Both C-6 astrocytoma cells and C-1300 neuroblastoma cells show increased glycogen synthesis in response to glucose in the medium with an accompanying increase in active glycogen synthase and decreased active phosphorylase. As glucose reaches a critical level in the medium (about 2 mM), glycogenolysis occurs. The amount of glycogen phosphorylase in the active form increases, and the amount of glycogen synthase in the active form decreases. Insulin will promote glucose entry into both cell types, and accelerates glycogenesis with increased synthase in the active form. All of these changes occur without alterations in cyclic AMP. However, in response to hormone administration (norepinephrine) cyclic AMP in the C-6 cells is elevated ten-fold, with consequent activation of phosphorylase, inactivation of synthase and breakdown of glycogen stores. Phosphodiesterase inhibitors, which prevent the breakdown of cyclic AMP, and adenyl cyclase stimulators can also stimulate cyclic AMP increases glycogenolysis in both C-6 astrocytoma and C-1300 neuroblastoma cells.

The alteration of NAD^+/NADH ratios and the metabolic pattern of normal and transformed fibroblasts has been studied in 5 cell lines. Removal of glucose increases the NAD^+/NADH ratio, within 5 minutes, and ATP declines to 75% of control after two hours. When galactose was the carbon source the NAD^+/NADH ratios resembled those in the absence of glucose; mannose or fructose produced intermediate ratio. There was an inverse relationship between the NAD^+/NADH ratio and growth for glucose, fructose and mannose.

In the Section on Cellular Neuropathology, the demyelinating disease research program has been continued and a new in vivo model has been developed to test the myelinotoxicity of CSF from patients with multiple sclerosis (MS) and other neurological diseases. It now has been used to test CSF from more than 50 patients. In double blind tests, the CSF samples from 60% of patients with a definite clinical diagnosis of active MS had positive myelinotoxic activity which seemed to correlate best with the severity of the disease, not with high CSF levels of either gamma globulin or total protein. The incidence of positive and borderline activity was much lower in CSFs from patients with possible MS and optic neuritis. Activity was negative in 85% of CSFs from a control group of patients with other neurological diseases. These results suggest that tadpole optic nerves are a useful, relatively simple in vivo model for investigating mechanisms of demyelination in multiple sclerosis. Only 0.5 ml. of unconcentrated CSF is needed for a test and the result is known in five days.

The Section on Neurocytology is concerned with the mechanisms and consequences of retrograde transport of protein within axoplasm. It has been established, during the past year, that horseradish peroxidase (HRP) injected into peripheral blood enters neuronal cell bodies surrounded by cerebral capillaries impermeable to the protein. The route of entry into the neuronal soma is not extracellular but rather intracellular. HRP readily crosses muscle capillaries to be pinocytosed by axon terminals from which the protein migrates retrogradely, by fast axoplasmic streaming, back to the parent cell body. These experiments were the first to show that circulating protein, unavailable to neurons from cerebral blood can actually enter the neurons from peripheral blood.

A proof of the transcellular route has been to ligate the hypoglossal nerve. HRP that enters the cell bodies of the XII nerve muscles does not enter those on the ligated side. These findings have been extended over the past few months, by demonstrating that the lack of protein on the ligated side is not only due to blockage of retrograde axonal transport but to a heightened enzymatic degradation of protein. Twelve hours after intravenous injection of HRP, the protein enters both hypoglossal nuclei. When, at this time, one nerve is ligated after the protein has entered the cell bodies, and 12 additional hours later, the brain is fixed, the cell bodies on the ligated side contain some but very little HRP. A retrograde reaction to nerve crush is an increase in number and enzymatic activity of lysosomes, reactions which account for our observations.

It has been claimed recently, that in situ neuronal cell bodies themselves can directly take up significant amounts of protein from the surrounding extracellular space. However, when we perfuse HRP through the cerebral ventricles, periventricular neurons, including those of the hypoglossal nucleus, become surrounded by protein. Even after 4 hours, very little if any protein is pinocytosed by the soma and dendrites. We are now examining, electron microscopically, which axoplasmic organelles are capable of transporting protein. Do vesicles, vacuoles or endoplasmic reticulum accumulate in the dammed portion of axoplasm on the distal side of a ligature?

Can lysosomes form in that portion of an axon separated from its soma by a ligature?

A second area of investigation is on the purported blood-brain barrier to lipids. We have recently found that polyunsaturated fatty acids (fa), readily visible in the electronmicroscope, when injected at high concentration into the carotid artery of rats, act as a soap and pass directly across the endothelial cell membrane of both extra- and intracerebral blood vessels. The fat droplets then penetrate the surrounding smooth muscle cells of the vessels and tend to aggregate next to mitochondria. Droplets also consistently enter the perinuclear cistern of various cell types but never the nucleus itself. The entry into axons appears to be confined primarily to the axoplasmic matrix.

A related project is to test the basic tenet that in frozen, fractured cell membranes, the nonparticle region is the lipid phase. By intercalating sterols such as cholesterol and ergosterol and other lipids including those in liposomes, into cell membranes of cerebral vessels and choroid plexus epithelium, the nonparticle area should increase. We are conducting these experiments in normal and stroke-prone rats to see whether lipids can be intercalated more readily into the cell membranes of the latter group.

In the Section on Functional Neuroanatomy, a major research effort is concerned with the structural basis of synaptic transmission. This project seeks to clarify the exact location and mechanism of synaptic transmission in the central and peripheral nervous system. It is carried out by examining synapses with the electron microscope to determine the influence of various functional states on their structure.

This year the Section published a method for capturing the fleeting structural details in functioning synapses by freezing them very rapidly, thereby avoiding the use of chemical fixatives and cryoprotective agents which alter profoundly the structure of synapses. By combining this method with the freeze-fracture technique it was possible to see how synaptic vesicles fuse with the surface of the synapse to discharge their contents. Also, particulate components of the synaptic vesicle wall, which are incorporated into the surface membrane during synaptic activity to be later recovered and incorporated into new synaptic vesicles were recognized. This finding of particle recycling confirms earlier work which showed that local recycling of synaptic vesicles replaces those lost during synaptic activity.

Freeze-fracturing is now being applied to see whether exocytosis and membrane recycling occur in different pharmacological types of synapses. So far, the results at synapses using glutamate (or aspartate) as a transmitter are consistent with the conclusions above. The effects of botulin toxin and black widow spider venom on synapses are also explored. So far, the results indicate that the toxin specifically blocks exocytosis, probably by preventing calcium ions from entering the nerve terminal, and that the venom lets calcium ions into excite exocytosis. One of the most interesting findings, which supports these conclusions, is that the venom can overcome the effect of the toxin, so that the normal distribution of exocytosis

reappears. The clinical usefulness of this finding is limited, however, unless a way could be found to return the exocytosis to control by nerve impulses arriving at the synapse.

The freeze-fracture technique has also yielded new information about the structure of the postsynaptic membrane. Particulate structures, thought to be receptor molecules within the postsynaptic membrane, appear to be specific for different types of synapse. Nicotinic receptors at excitatory cholinergic synapses always have the same structure, regardless of the species of animal examined, but presumed cholinergic synapses in the middle ear, which are thought to be inhibitory, have a different type of postsynaptic specialization. Conversely, neuromuscular synapses, which are excitatory, have different types of postsynaptic specializations depending on whether acetylcholine or glutamate is the transmitter. While more chemical types of synapses need to be examined, these studies indicate that identification of pharmacological types of synapses might be approached with anatomical techniques.

A second major project in the Section on Functional Neuroanatomy refers to permeability of cellular layers in the vertebrate nervous system. Unlike most regions of the brain, the median eminence lacks a blood-brain barrier (BBB) at the walls of its blood vessels. However, it is isolated from the cerebrospinal fluid (CSF) by a specialized ependyma which acts as a barrier between its parenchyma and the overlying CSF. Similarly, the dural covering of the brain lacks a BBB but embedded in the underlying arachnoid is a specialized "barrier layer" of cells between the dura and the CSF. This barrier layer of the arachnoid is missing at arachnoid villae where CSF is reabsorbed. The conclusion which emerges from these studies is that the brain and CSF are separated from the blood by a continuous layer of cells which act as a barrier to proteins. This barrier layer is absent at only a few locations for specific purposes such as reabsorption of CSF.

Barriers in the organ of Corti of the mammalian inner ear were the object of a collaborative study with the Laboratory of Neuro-Otolaryngology. The perilymph is separated from the endolymphic fluid by tight junctions between the apices of hair and supporting cells. The freeze-fracture technique was used to examine these junctions and they are more extensive and, presumably, "tighter" than in any other tissue studied so far. Their unique form also suggests that they might have functions other than to prevent mixing of endolymph and perilymph and, perhaps, are involved in the first steps in the sensing of mechanical displacements in the organ of Corti.

A third interest has been neurotrophic viruses, a project undertaken in collaboration with the Infectious Diseases Branch. We hoped to elucidate how neurotrophic viruses form in infected cells but also expected that infected cells would serve as good experimental subjects with which to develop methods to see how components of their membrane, such as viral antigens, are aggregated in special regions of the cell surface. For these purposes, techniques for seeing surface details as small as single protein molecules, and a way to tag these surface sites with antibodies were developed. These techniques made it possible to see how viral membrane is assembled and with this knowledge it

was possible to pinpoint the steps which are missing in chronic (slow) viral infections with such agents as the strain of measles virus which causes sub-acute sclerosing panencephalitis. Whether the same deficits are responsible for the development of slow virus infection in patients remains to be seen.

The Section on Experimental Neuropathology has been engaged in studies on cerebral fat emboli, cerebral siderosis, the effect of hormones on retro-grade reaction after severance of the facial nerve and on mast cells.

The role of fat emboli occurring in a variety of experimental and clinical conditions is widely disputed; in the opinion of some authors, they are incidental observations of no significance as cause of death, while others maintain that the presence of such emboli is an unequivocal sign of intra vitam circulation. The unusual demonstration of fat emboli in the brain and retina of normal animals fixed by perfusion could not be explained on the basis of current experience. By a repeated review of the manner in which the material was perfused, the source of the fat was finally found to be tissue fat which had accumulated on the cerebral surface during removal of the brain; fat emboli of such origin should be termed false fat emboli. For the emboli to lodge in the cerebral blood vessels, several conditions must coexist. When the brain is fixed by perfusion, the viscous blood is replaced by a liquid fixative, and if there are openings in the pia and blood vessels, the fat can be sucked or aspirated into the vascular channels as the consequence of stretching and constricting of blood vessels during manipulation of the brain. In support of this view, it was found experimentally that the emboli were more numerous when the brain was removed under ample covering of oil, and their formation was prevented when the brain was removed under continuous flow of water. Histologic differences between such emboli and intravital emboli were assessed in acute experiments with injection of oil. In order to prevent the introduction of connective tissue fat and to avoid diagnostic uncertainties, brains fixed by perfusion should be removed under flow of water and the optic nerve ligated whenever the question of fat emboli arises.

In subacute experiments with intravenous and intraperitoneal injection of oil, focal diffusion of iron was noted in the vicinity of tissue lesions. By this deposition of iron, the site of damaged blood-brain barrier is permanently marked, and conversely, in the absence of such changes, as in the brains with false emboli, it can be concluded that microcirculation has not been compromised and that the false emboli have not circulated intra vitam. The origin of the iron and the effect of diffused iron on tissue elements are being explored in different animals in which the hematologic status is being analyzed.

The effect of hormones on the reactive changes after severance of the facial nerve has been investigated in acute experiments with cortisone. This drug was found in the acute phase to accelerate the rate of degeneration and to delay the rate of recovery. A more prolonged effect on the rate of regeneration will be assessed microscopically in the brains of operated animals treated with this drug and allowed to survive the operation for several months. For the purpose of comparison, similarly operated animals

were treated with a thyroid hormone preparation, which is said to promote neuronal regeneration and functional recovery from peripheral nerve transection. A preliminary review of the microscopic sections disclosed that the acute morphologic reaction is of the same intensity as in nontreated animals. In order to estimate differences in the severity of neuronal loss, material from animals with prolonged survival is being prepared. In some instances, the animals were reoperated to establish whether treatment with any one of these hormones can be of benefit to neurons exposed to repeated insults.

Mast cells occur in the brains of most animals and tend to aggregate in large numbers in certain regions. Although they contain several biologically active substances, such as heparin, histamine and serotonin, their role in the nervous system is still unknown. A previous study demonstrated their transition from active to exhausted forms, as evidenced by progressive depletion of cytoplasmic material and pyknosis of nuclei. The nature of these changes will be elucidated with the electron microscope in a collaborative study with the Armed Forces Radiobiology Institute. This may provide a basis to evaluate changes in the functional capacity of the mast cells and to establish the degree of interaction between these cells and nervous tissue elements.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 201 NS 01885-06 LNNS																
PERIOD COVERED July 1, 1975 to June 30, 1976																		
TITLE OF PROJECT (80 characters or less) The Kinetics of Brain Glycogen																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																		
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">J. V. Passonneau</td> <td style="width: 30%;">Head, Sect. on Cellular Neurochem.</td> <td style="width: 20%;">LNNS NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>W. D. Lust</td> <td>Staff Fellow</td> <td>LNNS NINCDS</td> </tr> <tr> <td></td> <td>B. B. Mrsulja</td> <td>Visiting Scientist</td> <td>LNNS NINCDS</td> </tr> <tr> <td></td> <td>S. K. Crites</td> <td>Biologist</td> <td>LNNS NINCDS</td> </tr> </table>			PI:	J. V. Passonneau	Head, Sect. on Cellular Neurochem.	LNNS NINCDS	OTHER:	W. D. Lust	Staff Fellow	LNNS NINCDS		B. B. Mrsulja	Visiting Scientist	LNNS NINCDS		S. K. Crites	Biologist	LNNS NINCDS
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	B. B. Mrsulja	Visiting Scientist	LNNS NINCDS															
	S. K. Crites	Biologist	LNNS NINCDS															
COOPERATING UNITS (if any) None																		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences																		
SECTION Section on Cellular Neurochemistry																		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014																		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																
0.5	0.4	0.1																
SUMMARY OF WORK (200 words or less - underline keywords)																		
<p> The changes in <u>glycogen concentrations</u> during and following <u>ischemic episodes</u> were studied. <u>Mongolian gerbils</u> were made <u>ischemic</u> by occlusion of the left carotid artery. After either <u>1 or 3 hours</u> of occlusion, glycogen levels in the ischemic cortex were 10% of control. Following <u>recirculation</u>, glycogen levels were normal after 1 hour in both ischemic groups. After 1 week or recirculation, glycogen levels were twice normal concentrations in animals that had been ischemic 1 hour. In the <u>3 hour</u> ischemic group, <u>glycogen</u> concentrations increased to 170% of control after 5 hours of recirculation. In this group, glycogen was still elevated after 20 hours or 1 week of recirculation. </p>																		

Project Description:

Objectives: To further explore factors in the turnover, accumulation and disappearance of glycogen in the brain. To determine the effects of trauma, drugs, and altered physiological states such as hypothermia, ischemia, or drug administration on glycogen metabolism.

Methods Employed: Mongolian gerbils, 50-60 g were anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and unilateral ischemia was produced by occluding the left common carotid artery with an aneurysm clip. The animals with neurological symptoms were frozen after 1 or 3 hours of ischemia, or after 1 hour to 1 week of recirculation. The cerebral cortex was excised at -20° from the hemispheres ipsilateral or contralateral to the occluded artery. Sham-operated animals were also used as controls. The tissue was extracted in 0.03N HCl and the glycogen measured by an enzymic procedure.

Major Findings: The glycogen concentration in the contralateral cerebral cortex was essentially the same as that of sham-operated gerbils (19 nmoles/mg protein). Occlusion of the common carotid reduced the glycogen concentration to 1.5 or 1.8 nmoles/mg protein after 1 or 3 hours of ischemia. After 1 hour of recirculation, glycogen concentration appeared normal in both groups of animals previously made ischemic. In the 1 hour ischemic group glycogen appeared normal until after 1 week of recirculation. At this time, the glycogen was twice the normal concentration. After 3 hours of ischemia, and 5 hours of recirculation, cerebral glycogen was increased 170% over control values. After 20 hours and 1 week of circulation, the glycogen stores in this group were still elevated.

Significance to Biomedical Research and the Program of the Institute: The decreases in glycogen stores were comparable after 1 or 3 hours of ischemia. Following recirculation the restoration to normal values required 1 hour. However, in the 3 hour ischemic group, glycogen concentrations increased above normal after 5 hours of recirculation and persisted for 1 week. In the 1 hour ischemic group, the glycogen appeared normal until after 1 week of recirculation. The biochemical events following recirculation appear to reflect the severity of ischemic insult more accurately than those changes occurring during the ischemic period. Once the ischemic threshold is reached, the length of time required for the development of lesions is an inverse function of the length of the initial ischemic interval. Lesions appear earlier after longer periods of ischemia.

Proposed Course of the Project: Further studies will be made in the relationship of glycogen accumulation to ischemia and recirculation.

Publications: Lust, W. D., Passonneau, J. V., and Crites, S. K.: The measurement of glycogen in tissues by amylo- α -1,4- α -1,6-glucosidase after the destruction of preexisting glucose. Anal. Biochem. 68: 328-331, 1975.

Mrsulja, B. B., Lust, W. D., Mrsulja, B. J., Passonneau, J. V., and Klatzo, I.: Brain glycogen following experimental cerebral ischemia; biochemical evidence for maturation phenomenon. Experientia, 1976 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 NS 01942-05 LNNS</div>
PERIOD COVERED <div style="text-align: center; font-weight: bold;">July 1, 1975 to June 30, 1976</div>		
TITLE OF PROJECT (80 characters or less) <div style="text-align: center; font-weight: bold;">The Role of Cyclic AMP and Cyclic GMP in the Central Nervous System</div>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	W. D. Lust J. V. Passonneau H. J. Kupferberg W. D. Yonekawa	Staff Fellow Head, Section on Cell. Neurochem. Pharmacologist Pharmacologist
		LNNS NINCDS LNNS NINCDS EB NDP NINCDS EB NDP NINCDS
COOPERATING UNITS (if any) <div style="text-align: center; font-weight: bold;">Epilepsy Branch, Neurological Disorders Program, NINCDS</div>		
LAB/BRANCH <div style="text-align: center; font-weight: bold;">Laboratory of Neuropathology and Neuroanatomical Sciences</div>		
SECTION <div style="text-align: center; font-weight: bold;">Section on Cellular Neurochemistry</div>		
INSTITUTE AND LOCATION <div style="text-align: center; font-weight: bold;">NINCDS, NIH, Bethesda, Maryland 20014</div>		
TOTAL MANYEARS: <div style="text-align: center; font-weight: bold;">0.5</div>	PROFESSIONAL: <div style="text-align: center; font-weight: bold;">0.5</div>	OTHER: <div style="text-align: center; font-weight: bold;">0</div>
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> The levels of cyclic nucleotides were determined in both cerebellum and cerebral cortex following either pharmacological treatment or during electrically-induced <u>seizures</u>. Generally, <u>neurotropic agents</u> which either depress the CNS or protect against seizures decrease cerebellum <u>cyclic GMP</u> and agents which stimulate the CNS increase the cyclic GMP. <u>Cyclic AMP</u> is not pharmacologically responsive in either region. <u>Electroconvulsive shock</u> increases the levels of both cyclic nucleotides in both the cerebellum and the cerebral cortex. However, there is a temporal disparity in the cyclic nucleotide responses which favors the concept that the two cyclic nucleotides have separate and distinct roles in the CNS. </p>		

Project Description:

Objectives: To determine if either pharmacological or physiological alterations of brain metabolism would affect the steady state levels of cyclic AMP and/or cyclic GMP in vivo.

Methods Employed: Mice were rapidly frozen in liquid nitrogen at the appropriate times following treatment. The brains were removed at -25° , weighed and extracted in perchloric acid. The neutralized PCA extracts were used in all subsequent metabolic measurements. Cyclic GMP was measured by an enzymic-cycling technique or by the immunoassay method of Steiner, and cyclic AMP was measured by a radioactive binding assay.

Electroconvulsive shock (ECS) was applied by corneal electrodes at an intensity of 50 mA for a duration of 0.2 sec. The electroshock produces a convulsive response manifested by (a) a tonic extensor phase (0-15 sec.), (b) an intermittent clonic phase (15-30 sec.), and (c) a postictal depressive phase (> 30 sec.).

In the thermal experiments, the mice were rendered (a) hypothermic (20°) by forcing them to swim for 1-2 min. in a water bath followed by a 10 min. stay in a cold chamber, and (b) hyperthermic (41°) by placing them under a heat lamp. Rectal temperatures were monitored with a tele-thermometer.

Major Findings: The continuation of the work on electroconvulsive shock (ECS) and ischemia (produced by decapitation) confirmed our previous findings that the levels of cyclic AMP increase rapidly following both treatments in all areas of the brain tested. The levels of cyclic GMP in the cerebellum also increase almost 4-fold following ECS. The major difference between the cyclic nucleotide responses in the cerebellum is that the maximal change in cyclic AMP occurs at 15 seconds and precedes the cyclic GMP peak by approximately 45 seconds. Relating the cerebellar cyclic nucleotide responses to convulsive behavior, it is apparent that the major cyclic AMP changes occur during the excitable states (tonic, 0-15 sec. and clonic, 15-30 sec.), and cyclic GMP during the depressive state (postictal, greater than 30 sec.). Although the levels of cyclic nucleotides drop rapidly following their maximal ECS-induced changes, the restoration of cyclic AMP and cyclic GMP to control levels was not reached by 240 seconds. In addition, the cyclic AMP response to ECS differs in the cerebral cortex and the cerebellum. The cyclic AMP response in the cerebral cortex is 2-3 fold greater than that in the cerebellum and the peak response occurs at 45 sec. post-ECS or some 30 seconds after the cerebellar maximum. In contrast, the cyclic AMP increase following decapitation is greater in the cerebellum than in the cerebral cortex.

To determine what effect altered body temperature would have on cyclic nucleotides, mice were rendered hypothermic (20°) or hyperthermic (41°) as described in the methods. In hypothermic mice, the levels of cyclic AMP

were lower than normothermic values in both the cerebellum and the cerebral cortex. The levels of cyclic GMP were not affected. Further, the cyclic AMP increases following either decapitation or ECS were significantly reduced. In hyperthermic mice, both cyclic nucleotides were unchanged in NIH mice; however, the concentrations of cyclic GMP increased 2-fold in the DBA-2N strain of mice. Hypothermia delays the cyclic AMP response to ECS in the cerebral cortex. Although the peak levels resulting from ECS are approximately the same in both hypothermic and normothermic mice, the hypothermic maximum occurs at 240 sec. post-ECS, some 3 min. after the normothermic peak. From the measurements of ATP, phosphocreatin, glucose and phosphorylase a, post-ECS, it appears (as is the case with cyclic AMP) that hypothermia only affects the time course and not the magnitude of these metabolic changes.

In pharmacological studies, phenobarbital, papaverine and $MgSO_4$ significantly decreased the levels of cerebellar cyclic GMP. Phenobarbital (100 mg/kg IP) lowered cyclic GMP within 5 min. and the levels remained at 20% of control for up to 90 min. Of all the drugs tested, only chlorpromazine and trifluopromazine lowered cyclic AMP levels. Pretreatment with two drugs, amphetamine (10 mg/kg IP) which elevates GMP 2-fold, or phenobarbital (20 mg/kg IP) which decreases cyclic GMP 50%, had no effect on the ECS-induced increase in cyclic nucleotides, in spite of significant alterations in overt convulsive behavior.

The anti-convulsant agents, phenytoin, clonazepam (C) and dipropylacetic acid (DPA) decreased cerebellar cyclic GMP levels by more than 50%. Conversely, the convulsant agents, isoniazid (INH), pentylenetetrazol (PTZ) and theophylline increased the cyclic GMP levels by at least 3-fold. And in combination, the convulsant-induced elevation of cyclic GMP was inhibited by the anti-convulsant agents. Specifically, DPA and C blocked the INH-induced increase in cyclic GMP, and C blocked the PTZ-induced increase. Thus, these anti-convulsant agents were not only able to block the chemically-induced seizures, but also the increase in cerebellar cyclic GMP.

A time course following 400 mg/kg IP of DPA provides additional evidence for a relationship between anti-convulsant activity and cerebellar cyclic GMP levels. Anti-convulsant activity here was defined as the ability to abolish tonic extension following ECS. The levels of cyclic GMP were depressed at 1/2 hour and 1 hour after injection when more than 60% of the mice were protected against seizures. As anti-convulsant activity diminished at 2 and 4 hours, the cyclic GMP returned to control levels. So, the lowered cyclic GMP levels coincided temporally with the anti-convulsant activity.

Additional studies with anti-convulsants demonstrated that all the agents with the exception of Diamox depressed cerebellar cyclic GMP levels. Four anti-convulsants were found to have no effect on the levels of cerebral cortical cyclic GMP. In contrast, the convulsants, isoniazid and pentylene-tetrazol, increased the levels of cyclic GMP in the cerebral cortex, as well as the cerebellum. ECS also increased the levels of cyclic GMP some 5-fold in the cerebral cortex.

Mice pretreated with 20 mg/kg of phenytoin exhibited a clonic-type convulsion instead of the tonic extension normally observed following ECS. In spite of the alterations in convulsive behavior, the ECS-induced changes in cyclic nucleotides were only minimally affected by the agent. The cyclic GMP elevation in the cerebellum was reduced by 50%, whereas the cyclic AMP changes and the cyclic GMP increase in the cerebral cortex exhibited little or no change.

Utilizing a more sensitive cyclic GMP assay, it was reconfirmed that cerebellar cyclic GMP levels were about an order of magnitude greater than those in the cerebral cortex. Decapitation (anoxia) did not elevate cyclic GMP in either region as was previously reported by other investigators. Pretreatment with ethanol (2 g/kg) reduced cyclic GMP in both cerebellum and cerebral cortex. Decapitation (15 sec.) along with ethanol pretreatment produced a greater decrease in cyclic GMP than that observed with ethanol alone. Further, ethanol which had no effect on the levels of cyclic AMP reduced the decapitation-induced increase in cyclic AMP.

Using four different fixation techniques, the levels of cyclic AMP were lowest in the microwave irradiated by brain with increasing amounts in brains which were freeze-blown, frozen by immersion and frozen following decapitation. In comparison, the degree of anoxia as determined by the changes in ATP, phosphocreatine and lactate was lowest in the freeze-blown brain, with increasing anoxia in brains which were frozen by immersion, irradiated and frozen following decapitation.

Significance to Biomedical Research and the Program of the Institute: Both the ECS and pharmacological data favor the argument that cyclic AMP and cyclic GMP are independently regulated in vivo. The possibility of a direct relationship between elevated cyclic GMP and depression seems remote, since pharmacologically-induced depression either reduced or had no effect on cyclic GMP levels. There is an increasing amount of evidence favoring an association between either lowered cyclic AMP or elevated cyclic GMP and CNS excitation; and between elevated cyclic AMP or lowered cyclic GMP and CNS depression. Thus, neural events following a number of neurotropic agents or such treatments as ECS or brain injury might be defined by the cyclic nucleotide profiles. From our studies with pharmacological agents, the levels of cerebellar cyclic GMP appear to reflect a behavioral spectrum from depression to convulsion; CNS depressants and anti-convulsant agents lower cyclic GMP while convulsant agents elevate cyclic GMP. Thus, cyclic GMP in the cerebellum may serve as a molecular indicator of the degree of CNS excitability, and therefore may be useful in our understanding of the mechanism of action of a variety of neurotropic drugs.

The ECS- and anoxia-induced changes in brain cyclic AMP exhibit regional variation. Thus, while the cerebellum is more sensitive to the stimulus of anoxia, the cerebral cortex is more responsive to the electrical stimulus. Although hypothermia does slow metabolic events in the brain, it is apparent that only the temporal relationships and not the magnitude of response are affected.

The cyclic nucleotide elevation following ECS has been attributed by others to be a result of the apnea-induced anoxia which occurs during tonic extension. The absence of a cyclic GMP increase in either cortex or cerebellum 15 sec. after decapitation would tend to refute this explanation. Further, reduction of the anoxic period during tonic extension by phenytoin had only a slight effect on the cyclic nucleotide response. These results would tend to decrease the likelihood that anoxia is the major stimulus to the cyclic nucleotide increases after electroshock.

Since cyclic AMP increases rapidly in the brain following decapitation, low cyclic AMP levels have been equated with rapid fixation. However, following microwave irradiation low levels of cyclic AMP were obtained although the irradiated brains were anoxic by other criteria. Of the fixation methods tested, freeze-blowing and freezing intact most closely approximate the in vivo state.

Proposed Course of the Project: Presently, the primary emphasis is on the relationship of cerebellar cyclic GMP to the different types of anti-convulsant agents, and their different anti-convulsant activities (anti-pentylenetetrazol, anti-ECS, etc.). Also, more extensive studies on the seizure-dependent rise of cyclic nucleotides whether chemically- or electrically-induced are being undertaken in other regions, as well as the cerebellum.

Publications: Lust, W. D., Goldberg, N. D., and Passonneau, J. V.: Cyclic nucleotides in murine brain: The temporal relationship of changes induced in adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate following maximal electroshock or decapitation. J. Neurochem. 26: 5-10, 1976.

Lust, W. D. and Passonneau, J. V.: Cyclic nucleotides in murine brain: Effect of hypothermia on adenosine 3',5' monophosphate, glycogen phosphorylase, glycogen synthase and metabolites following maximal electroshock or decapitation. J. Neurochem. 26: 11-16, 1976.

Lust, W. D., Kupferberg, H. J., Passonneau, J. V., and Penry, J. K.: On the mechanism of action of sodium valproate: The relationship of GABA and cyclic AMP levels to anti-convulsant activity. Epilim Symposium, Nottingham, England, 1976 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02006-04 LNNS																
PERIOD COVERED July 1, 1975 to June 30, 1976																		
TITLE OF PROJECT (80 characters or less) Regulation of Metabolism in Glioma and Neuroblastoma Cell Lines																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																		
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">J. V. Passonneau</td> <td style="width: 40%;">Head, Sect. on Cellular Neurochem.</td> <td style="width: 20%;">LNNS NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>W. D. Lust</td> <td>Staff Fellow</td> <td>LNNS NINCDS</td> </tr> <tr> <td></td> <td>S. K. Crites</td> <td>Biologist</td> <td>LNNS NINCDS</td> </tr> <tr> <td></td> <td>J. P. Schwartz</td> <td>Staff Fellow</td> <td>LNNS NINCDS</td> </tr> </table>			PI:	J. V. Passonneau	Head, Sect. on Cellular Neurochem.	LNNS NINCDS	OTHER:	W. D. Lust	Staff Fellow	LNNS NINCDS		S. K. Crites	Biologist	LNNS NINCDS		J. P. Schwartz	Staff Fellow	LNNS NINCDS
PI:	J. V. Passonneau	Head, Sect. on Cellular Neurochem.	LNNS NINCDS															
OTHER:	W. D. Lust	Staff Fellow	LNNS NINCDS															
	S. K. Crites	Biologist	LNNS NINCDS															
	J. P. Schwartz	Staff Fellow	LNNS NINCDS															
COOPERATING UNITS (if any) None																		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences																		
SECTION Section on Cellular Neurochemistry																		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014																		
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MANYEARS:</td> <td style="width: 33%;">PROFESSIONAL:</td> <td style="width: 33%;">OTHER:</td> </tr> <tr> <td style="text-align: center;">0.7</td> <td style="text-align: center;">0.6</td> <td style="text-align: center;">0.1</td> </tr> </table>			TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	0.7	0.6	0.1										
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																
0.7	0.6	0.1																
SUMMARY OF WORK (200 words or less - underline keywords) Studies are being carried out on 2 cell lines in culture. C-1300 <u>neuroblas-</u> <u>toma</u> cells, and C-6 <u>astrocytoma</u> cells are used to investigate differences, if any between the <u>metabolism</u> of cells of <u>glial</u> and <u>neural</u> origin. The regulation of <u>cyclic AMP</u> and <u>cyclic GMP</u> concentrations in the cells, and the fluctuation of these compounds in response to <u>hormones</u> and/or <u>drugs</u> is under investigation. C-6 cells respond to <u>norepinephrine</u> , and C-1300 cells respond to <u>prostaglandin E</u> , in that adenyl cyclase is activated and cyclic AMP con- centrations increase. Both cell lines have <u>phosphodiesterase</u> activities which are specific for the degradation of the 2 cyclic nucleotides, and both enzyme activities can be induced. The effect of <u>thiamine deficiency</u> or metabolites and thiamine-linked enzymes has been investigated in both cell lines. A study of the effects of <u>glucose deprivation</u> and/or anaerobiasis has also been undertaken.																		

Project Description:

Objectives: To investigate the regulation of metabolism in a glioma and a neuroblastoma cell line.

Methods Employed: The cells are being grown in a CO₂ incubator in the laboratory. Homogenates of the cells are analyzed for various metabolites, such as cyclic AMP, cyclic GMP, and pyridine nucleotides, as well as for enzymes such as the adenylate and guanylate cyclases, cAMP phosphodiesterase and glycogen synthetase and phosphorylase. All of these analytical methods have been used in the laboratory on whole brain and are thus easily adaptable for use with the cells. In addition, the effect of various agents on the morphology of the cells will be undertaken.

Major Findings: The glioma cells contain an adenylate cyclase which is responsive to catecholamines such as norepinephrine. Activation of the cyclase by norepinephrine elevates the intracellular cyclic AMP level, resulting in the breakdown of glycogen due to inactivation of the glycogen synthetase and activation of the phosphorylase (Browning, E. T., Schwartz, J. P., and Breckenridge, B. M.: Molec. Pharmacol. 10: 162, 1974). The expression of the norepinephrine receptor has been shown to require cell contact. Activity of the cyclic AMP phosphodiesterase can also be regulated, by growth of the cells in either dibutyryl cAMP or in bromo-deoxy-uridine, an agent which causes dedifferentiation (Schwartz, J. P., Morris, N. R., and Breckenridge, B. M.: J. Biol. Chem. 248: 2699, 1973). Treatment of the glioma cells with norepinephrine not only results in short-term effects, but also causes an induction of the phosphodiesterase. The induction has been shown to be mediated by cyclic AMP via the β -receptor and to require new protein synthesis.

The effects of other hormones and drugs, such as depolarizing agents, on the metabolism of both cyclic AMP and cyclic GMP, have also been studied. In addition, both cell lines have been shown to contain individual phosphodiesterases specific for the 2 cyclic nucleotides, which can be independently induced.

In addition to cyclic AMP metabolism, a study of the energy metabolism of both cell lines has been carried out. The kinetics of glucose uptake, of glycogen breakdown, and of pyruvate and lactate efflux and uptake have been studied. The effect of thiamine deficiency, brought about by growth without thiamine or in the presence of the anti-thiamine drug pyriethamine, on the above parameters as well as thiamine levels and thiamine-requiring enzymes has also been examined. In addition, the effects of the local anesthetic, tetracaine, on this system have been studied.

Significance to Biomedical Research and the Program of the Institute: A study of the regulation of metabolism in the brain is complicated by the presence of several cell types and the inability to determine in which cells metabolic alterations are occurring. Such studies are facilitated by the

use of the two distinct cell lines, the glioma (rat C-6 line) and the neuroblastoma (mouse C-1300 line), because regulation can be studied in each cell line separately. Until these cell lines became available, for example, it had not been realized that glial cells might be hormone-responsive. Such results suggest that the glial cells may have a greater involvement in brain function than the supportive one originally envisaged. Although these two cell lines are tumor cells, they offer a first approach to the problem of regulation of metabolism in the brain, which might be followed up in normal brain cells when the isolation techniques for glial and neuronal cells have been perfected.

Proposed Course of the Project: A thorough investigation of the regulation of cyclic AMP and cyclic GMP levels, of other energy-related parameters, and of pyridine nucleotide ratios will be undertaken, in both cell lines. Studies on ion transport are also planned, as are morphological studies of the effects of various agents and growth conditions. The effects of glucose deprivation and/or anaerobiasis on both cell lines is being investigated. Further studies related to the effect of thiamine deficiency on thiamine-dependent enzymes and the pentose shunt are planned.

Publications: Lust, W. D., Schwartz, J. P., and Passonneau, J. V.: Glycolytic metabolism in cultured cells of the nervous system. I. Glucose transport and metabolism in the C-6 glioma cell line. Molec. Cell. Biochem. 8: 169-176, 1975.

Schwartz, J. P., Lust, W. D., Lauderdale, V., and Passonneau, J. V.: Glycolytic metabolism in cultured cells of the nervous system. II. Regulation of pyruvate and lactate metabolism in the C-6 glioma cell line. Molec. Cell. Biochem. 9: 67-72, 1975.

Schwartz, J. P., Lust, W. D., Shirazawa, R., and Passonneau, J. V.: Glycolytic metabolism in cultured cells of the nervous system. III. The effects of thiamine deficiency and pyriithiamine on the C-6 glioma and C-1300 neuroblastoma cell lines. Molec. Cell. Biochem. 9: 73-78, 1975.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02140-02 LNNS						
PERIOD COVERED July 1, 1975 to June 30, 1976								
TITLE OF PROJECT (80 characters or less) Regulation of the NAD ⁺ /NADH and Metabolic Pattern of Normal and Transformed Fibroblasts								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 33%;">PI: J. P. Schwartz</td> <td style="width: 33%;">Staff Fellow</td> <td style="width: 33%;">LNNS NINCDS</td> </tr> <tr> <td>OTHER: G. S. Johnson</td> <td>Research Chemist</td> <td>LMBGY NCI</td> </tr> </table>			PI: J. P. Schwartz	Staff Fellow	LNNS NINCDS	OTHER: G. S. Johnson	Research Chemist	LMBGY NCI
PI: J. P. Schwartz	Staff Fellow	LNNS NINCDS						
OTHER: G. S. Johnson	Research Chemist	LMBGY NCI						
COOPERATING UNITS (if any) Laboratory of Molecular Biology, National Cancer Institute								
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences								
SECTION Section on Cellular Neurochemistry								
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014								
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0.0						
SUMMARY OF WORK (200 words or less - underline keywords) <p> The changes in <u>NAD⁺/NADH ratios</u> during cell growth in <u>normal and transformed cells</u> has been investigated. In the presence of <u>glucose</u> that ratio is 2 to 3, removal of glucose increases the ratio 4-fold, as does the substitution of <u>galactose</u> for glucose. No other <u>hexose</u> or carbon source can substitute for glucose completely for growth. The <u>redox state</u> varies widely dependent on the hexose used as the carbon source. There are striking changes in <u>cell morphology</u> associated with different sugars. Differences in rate of <u>sugar transport</u> could not explain these differences; nor could the activity of hexokinase explain the differences observed between <u>fructose</u> and <u>glucose</u>. </p>								

Project Description:

Objectives: To investigate the factors which regulate the NAD^+/NADH ratio and the metabolic pattern in normal and transformed cell lines.

Methods Employed: The cells are grown in tissue culture. Cell extracts are analyzed for various metabolites such as NAD^+ , NADH , ATP and cyclic AMP by a variety of fluorometric and immunological methods.

Major Findings: The cells are extremely sensitive to the presence and concentration of glucose in the medium. Five minutes after removal of glucose, the NAD^+/NADH ratio increases 3-4-fold, followed by a much more gradual decline in ATP (20% fall in 2 hrs.). Readdition of glucose restores the redox state within 5 seconds. No other sugar can substitute for glucose completely for growth: the redox state of cells grown on other sugars also ranges widely. For example, the NAD^+/NADH ratio in all cell lines tested was 2-3 in the presence of glucose, but 10-15 when galactose was substituted. There are no apparent changes in cyclic nucleotide metabolism related to these metabolic changes, but there are striking changes in cell morphology. Differences in the rates of sugar transport cannot explain these differences.

Significance to Biomedical Research and the Program of the Institute: The pyridine nucleotides (NAD^+ and NADH) are involved in many of the oxidation-reduction reactions of a cell. The ratio of NAD^+ to NADH is therefore a basic indicator of the metabolic state and growth properties of the cells. The differences in the ratio found between normal and tumor cells must therefore reflect the metabolic alteration in transformed cells. The ability to regulate this ratio in confluent transformed cells might thereby allow restoration to the growth and metabolic state of normal cells. Alteration of the glucose level or of the sugar source available may be one key to such regulation.

Proposed Course of the Project: This project has been terminated.

Publications: Johnson, G. S. and Schwartz, J. P.: Effects of sugars on the physiology of cultured fibroblasts. Exp. Cell Res. 97: 281-290, 1976.

Schwartz, J. P. and Johnson, G. S.: Metabolic effects of glucose deprivation and of various sugars in normal and transformed fibroblast cell lines. Arch. Biochem. 173: 237-245, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02141-02 LNNS												
PERIOD COVERED July 1, 1975 to June 30, 1976														
TITLE OF PROJECT (80 characters or less) Glycogen Metabolism in Glioma and Neuroblastoma Cell Lines														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">J. V. Passonneau</td> <td style="width: 30%;">Head, Sect. on Cellular Neurochem.</td> <td style="width: 20%;">LNNS NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>W. D. Lust</td> <td>Staff Fellow</td> <td>LNNS NINCDS</td> </tr> <tr> <td></td> <td>S. K. Crites</td> <td>Biologist</td> <td>LNNS NINCDS</td> </tr> </table>			PI:	J. V. Passonneau	Head, Sect. on Cellular Neurochem.	LNNS NINCDS	OTHER:	W. D. Lust	Staff Fellow	LNNS NINCDS		S. K. Crites	Biologist	LNNS NINCDS
PI:	J. V. Passonneau	Head, Sect. on Cellular Neurochem.	LNNS NINCDS											
OTHER:	W. D. Lust	Staff Fellow	LNNS NINCDS											
	S. K. Crites	Biologist	LNNS NINCDS											
COOPERATING UNITS (if any) None														
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences														
SECTION Section on Cellular Neurochemistry														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014														
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.1	OTHER: 0.3												
SUMMARY OF WORK (200 words or less - underline keywords) <p> The <u>regulation</u> of <u>glycogen metabolism</u> has been studied in <u>C-6 astrocytoma</u> and <u>C-1300 neuroblastoma</u> cell lines in culture. There appear to be two principal modes of <u>controlling glycogen synthesis</u> and breakdown. The <u>concentrations</u> of <u>glycogen</u> in the cells, and the amount of <u>glycogen phosphorylase</u> and <u>glycogen synthase</u> in the active forms can be regulated by the <u>available glucose supply</u>; however, there is a <u>maximum level of glycogen</u> which is not exceeded even in the presence of greatly increased glucose supply. When glucose in the medium falls below a critical level, phosphorylase is activated and glycogenolysis occurs. Substances which cause <u>cyclic 3',5'-AMP</u> to be elevated in the cells also stimulate glycogenolysis. In <u>C-6 glioma cells</u>, <u>norepinephrine</u> is effective; in C-1300 neuroblastoma cells, <u>prostaglandin E</u>, is effective. Both cell lines respond to the <u>phosphodiesterase inhibitor</u>, isobutyl methylxanthine, and the <u>adenyl cyclase stimulator</u> adenosine. </p>														

Project Description:

Objectives: To determine the factors regulating glycogen metabolism in glioma and neuroblastoma cell lines.

Methods Employed: Two cloned cell lines, C-6 astrocytoma cells (rat brain) and C-1300 neuroblastoma cells (mouse cord) are grown in a CO₂ incubator. Cells are rapidly fixed by freezing in liquid nitrogen to preserve the in vivo state. Homogenates of the cells are analyzed for total glycogen phosphorylase and total glycogen synthase, as well as the per cent of those enzymes which are in the a or active form. Extracts of cells grown under identical conditions are analyzed for glycogen, glucose, glucose 6-P, uridine diphosphoglucose and cyclic 3',5'-AMP (cyclic AMP) content. The two cell lines have been investigated with regard to response to available energy sources in the medium, as well as the influence of phosphodiesterase inhibitors, stimulators of adenylyl cyclase, and insulin.

Major Findings: The concentration of glycogen in C-6 glioma cells and C-1300 neuroblastoma cells is regulated in part by available energy sources, in this case glucose. However, there is a maximum level of glycogen which is not exceeded, even if the glucose concentration is increased from 5 to 50 mM in the medium. The rate of formation and concentration of glycogen in the cells are the same in either case. The peak level of glycogen in C-6 glioma cells is twice that of the neuroblastoma cells; the concentrations are 100-120 and 40-50 nmoles/mg protein in the C-6 and C-1300 cells, respectively. Similarly, the amount of glycogen synthase in the C-6 cells is twice that of the C-1300 cells, and the glycogen is accumulated faster in the C-6 cells. If insulin is added in the presence of glucose, intracellular concentrations of glucose increase; the amount of glycogen phosphorylase, the degradative enzyme, in the active form is decreased; the amount of glycogen synthase (the synthetic enzyme) in the active form is increased; and glycogen stores increase. All of these changes, which are related to available nutrients, occur without alterations in cyclic AMP concentrations in the cells.

However, the cells will respond to a variety of stimuli; certain hormones, phosphodiesterase inhibitors, or stimulators of adenylyl cyclase, cause intracellular concentrations of cyclic AMP to increase. In the case of norepinephrine, cyclic AMP increases more than 10-fold in C-6 cells and phosphorylase is converted primarily to the active form (4-fold increase), synthase to the inactive form and glycogenolysis occurs. Norepinephrine had no effect on C-1300 neuroblastoma cells. Prostaglandin E₁ was effective in raising cyclic AMP concentrations in C-1300 neuroblastoma cells. Isobutyl methylxanthine, a phosphodiesterase inhibitor, increased cyclic AMP in both cell lines, with consequent phosphorylase activation and degradation of glycogen. Adenosine, an activator of adenylyl cyclase, potentiated the effect of isobutyl methylxanthine in C-6 astrocytoma cells.

Significance to Biomedical Research and the Program of the Institute:

It has been demonstrated in these two cell lines of neural origin that regulation of glycogen metabolism can be exerted by regulation of supply of glucose. Previously, in mammalian systems, we showed that brain glycogen would increase if blood glucose levels were maintained at high concentrations, and if insulin were present. No glycogen increases in the brain could be demonstrated in alloxan diabetic mice, although blood glucose levels were 10-fold greater than normal. The demonstration of insulin effects in neural tissue is of great importance and has not previously been shown without ambiguity. In addition, the response of the cells to hormones and drugs has shown that the cyclic AMP activating system for phosphorylase can be controlled by a variety of agents. The response of the glycogen synthesizing and degrading system is thus under two modes of control which permits glycogen to be stored under ordinary conditions, and mobilized in response to stimulatory agents.

Proposed Course of the Project: Further studies are being made to determine the control of the amount of glycogen synthase, which also varies in response to the glucose in the medium. When glucose was increased 10-fold total synthase activity decreased to one-fourth the activity in the presence of 5 mM glucose. Whether this decrease in activity is due to protein degradation, "extra phosphorylation" or steric hindrance by the macromolecular glycogen will be investigated.

Publications: Passonneau, J. V. and Crites, S. K.: Regulation of glycogen metabolism in astrocytoma and neuroblastoma cells in culture. J. Biol. Chem. 251: 2015-2022, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02142-02 LNNS
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Biochemical Changes During Both Ischemia and the Recovery Following Ischemia		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	W. D. Lust M. Kobayashi B. B. Mrsulja B. J. Mrsulja J. P. Schwartz J. V. Passonneau	Staff Fellow Visiting Fellow Visiting Scientist Visiting Fellow Staff Fellow Head, Sect. on Cell. Neurochem.
		LNNS NINCDS LNNS NINCDS LNNS NINCDS LNNS NINCDS LNNS NINCDS LNNS NINCDS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cellular Neurochemistry		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 2.4	PROFESSIONAL: 2.4	OTHER: 0
SUMMARY OF WORK (200 words or less - underline keywords) <p> Various biochemical parameters were investigated in the gerbil cerebral cortex both during and after occlusion of the common carotid artery. <u>Ischemia</u> produced large changes in the energy metabolites, <u>cyclic nucleotides</u> and certain neurotransmitters, but had little or no effect on enzyme activities with the exception of protein kinase. From the data on the brain metabolites, there does not seem to be a major secondary change in energy metabolites for up to 6 hours of ischemia, but there is a gradual time-dependent change in the levels of putative neurotransmitters during the entire 6 hours of ischemia. <u>Recovery</u> from the ischemic insult was marked by a rapid restoration of energy metabolites, a short-term large increase in cyclic AMP, and a significant reduction in enzyme activities of Na^+, K^+-activated ATPase and adenylate cyclase. The results on enzyme and metabolites seem to indicate that the recovery process is dependent on the intensity of the ischemic insult and that recovery is more than a mere reversal of the ischemic-induced events. The pathological significance of these ischemia-related biochemical events are currently under investigation. </p>		

Project Description:

Objectives: To determine the alteration in enzymes, metabolites and putative neurotransmitters during long-term unilateral ischemia in the gerbil cerebral cortex; and, further, to monitor the recovery of these metabolites following a period of ischemia.

Methods Employed: Mongolian gerbils were anesthetized and the left common carotid artery was looped with a suture. As the gerbils emerged from the anesthesia, the artery was ligated. If the gerbils exhibited positive neurological symptoms, they were frozen in liquid nitrogen at various periods following ligation.

The cerebral cortex was removed at -20° and extracted in perchloric acid. The left hemisphere served as the ischemic side and the right as the control. ATP, P-creatine, glucose, glycogen, glutamate, citrate and gamma aminobutyric acid (GABA) were determined enzymically. Cyclic GMP was measured by immunoassay and cyclic AMP by the protein binding method. Dopamine, norepinephrine and 5-hydroxytryptamine were measured fluorometrically.

In the recovery studies, the carotid artery was occluded with a clip for the appropriate time and then released. At various times following release, the gerbils were frozen and brains removed as described above.

Major Findings: By most of the criteria previously established in the decapitated mouse brains, the left cerebral cortex following the ligation of the left common carotid artery is in fact ischemic. From 1/2 to 6 hours after ligation, the ATP, P-creatine, glycogen and glucose were decreased by 60% or more and remained low for the entire period. The right or control cerebral cortex was essentially the same as a sham control up to 6 hours.

Cyclic AMP in the ischemic side increased 7-fold to a maximum at 2 hours and thereafter decreased to control values by 6 hours. GABA increased with time in the ischemic cerebral cortex to a maximum 5-fold greater than control at 6 hours. In addition, putative neurotransmitters, dopamine, norepinephrine and 5-hydroxytryptamine decreased with increasing time of ligation. The initial decrease in dopamine and norepinephrine occurred within 30 minutes while that for 5-hydroxytryptamine occurred only after 3 hours of ischemia.

In the recovery studies, the metabolites were measured 5 minutes, 1, 5 and 20 hours after either 1 or 3 hours of ischemia. All the metabolites measured were essentially back to control levels by 1 hour of recovery. Even though cyclic AMP was restored by 1 hour, this cyclic nucleotide increased an additional 5-fold over the already elevated cyclic AMP levels after 5 minutes of recovery.

The effect of short-term ischemia were investigated utilizing bilateral ligation of the gerbil common carotid arteries. The levels of ATP, P-creatine, glucose and glycogen were maximally depressed after 1 minute of ischemia. The cyclic AMP levels increased 19-fold after 1 minute of ischemia and thereafter decreased to a level approximately 5-fold greater than control at 5 minutes of ischemia. Thus, the time course of cyclic AMP changes during ischemia is marked by an early large rise, followed by a drop of cyclic AMP to a plateau some 5-fold greater than control which persists for up to 2 hours and finally by a gradual decrease to control levels at 6 hours of ischemia. Recovery of ATP, P-creatine and glucose after both 1 and 5 minutes of ischemia was essentially complete with 5 minutes of recirculation. The large post-ischemic increase in cyclic AMP was observed at 5 minutes of recirculation following 5 but not 1 minute of ischemia. Theophylline pretreatment markedly reduced the ischemic but not the post-ischemic induced increase in cyclic AMP. While the cerebellum was not anoxic as indicated by the levels of ATP and P-creatine, there were small but consistent changes in the levels of the cyclic nucleotides and GABA. Both GABA at 5 minutes and cyclic AMP at 1 minute increased; whereas, cyclic GMP decreased at both 1 and 5 minutes of carotid occlusion. During recovery, the cyclic GMP levels decreased further to 24% of control.

The effects of ischemia on the activities of adenylate cyclase (A.C.), protein kinase and ATPase were investigated. Particulate cyclic AMP-dependent protein kinase of all the enzymes tested was the only one to decrease during ischemia. While the activities of Na^+ - K^+ -activated ATPase and A.C. were unaffected during ischemia, the activities of both enzymes decreased to 50% of the right side (control) value after 5 hours of recirculation. The control level of ATPase activity was restored after 20 hours of recirculation, while that for A.C. took up to 1 week of recirculation. Four other enzymes, including phosphodiesterases, soluble protein kinase and Mg-dependent ATPase, were unaffected during and after ischemia.

Significance to Biomedical Research and the Program of the Institute:

The gerbil model for unilateral ischemia permits the investigation of the long term effects of ischemia. From the measurement of the energy metabolites, the ischemic state is established by 30 minutes and remains for as long as the artery is ligated. The lowering of cyclic GMP and the elevation of both cyclic AMP and GABA all indicate the depressed state of the cerebral cortex. The reason for the decrease of cyclic AMP following peak levels at 2 hours is not clear, but may indicate a secondary stage of ischemic damage.

The rapid recovery following 1 and 3 hours of ischemia in spite of histochemical evidence of brain damage suggests that a major portion of the cortical cells do remain viable even after 3 hours of ischemia.

While the total restoration of the metabolites (1 h post-ischemic values) only appears to be compromised after 6 h of ischemia, the rate of recovery (5 m post-ischemic values) decreases with increasing periods of ischemia. Indications of a diminished rate of recovery as reflected by the ATP levels developed between 5 and 30 minutes of an ischemic insult.

The large changes in cyclic nucleotides probably reflect the severe biochemical perturbations which occur in the brain both during and after ischemia. It is quite evident that certain events occur in the ischemic cerebral cortex between 1 and 5 m of ischemia which permit the large post-ischemic accumulation of cyclic AMP observed at 5 m of recirculation after 5 but not 1 m of ischemia. The current thinking is that certain adenylate cyclase agonists are being liberated during the first five minutes of ischemia but that their expression is not manifested (in terms of elevated cyclic AMP) because of the low levels of the substrate for adenylate cyclase, ATP. Upon recirculation, the ATP levels are rapidly regenerated and in the presence of the available agonists, a burst of cyclic AMP production ensues. Thus, a proper temporal relationship between the loss of ATP and the liberation of adenylate cyclase agonists is critical to the cyclic AMP accumulation during recirculation. Theophylline is a potent anti-adenosine agent besides being a phosphodiesterase inhibitor. The fact that theophylline reduced the ischemic but not the post-ischemic rise in cyclic AMP indicates that (1) two different agonists may be involved and (2) adenosine may be the stimulus to the cyclic AMP accumulation during ischemia. Cyclic GMP levels decrease during ischemia and remain low during recirculation except for 1 and 5 m of recirculation when there is a 4-fold increase. Based on electrophysiological results from other laboratories, it would appear that neuronal excitability is reduced by cyclic AMP and increased by cyclic GMP. Therefore, the elevated cyclic AMP levels and the reduced cyclic GMP levels during and after ischemia would collectively have an inhibitory influence on cortical excitability.

The pathological significance of the post-ischemic rise in cyclic AMP to the recoverability of the ischemic cortex is presently unclear and is currently under investigation.

Proposed Course of the Project: The major emphasis is presently on the biochemical characteristics of recovery. In addition, the nature of the large recovery induced increase in cyclic AMP after short-term ischemia will be investigated more extensively.

Publications: Lust, W. D., Mrsulja, B. B., Mrsulja, B. J., Passonneau J. V., and Klatzo, I.: Putative neurotransmitters and cyclic nucleotides in prolonged ischemia of the cerebral cortex. Brain Res. 98: 394-399, 1975.

Mrsulja, B. B., Lust, W. D., Mrsulja, B. J., Passonneau, J. V., and Klatzo, I.: Brain glycogen following experimental cerebral ischemia in gerbils. Experientia, 1976 (in press).

Mrsulja, B. B., Lust, W. D., Mrsulja, B. J., Passonneau, J. V., and Klatzo, I.: Post-ischemic changes in certain metabolites following prolonged ischemia in the gerbil cerebral cortex. J. Neurochem., 1976 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02191-01 LNNS
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) GABA Shunt Metabolites and Enzymes in Mouse Brain and Cells in Culture		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: J. V. Passonneau OTHER: W. D. Lust S. K. Crites	Head, Sect. on Cell. Neurochem. Staff Fellow Biologist	LNNS NINCDS LNNS NINCDS LNNS NINCDS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cellular Neurochemistry		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.4	OTHER: 0.5
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> <u>GABA shunt metabolites</u> and <u>enzymes</u> have been measured in mouse brain and <u>glial</u> and <u>neuronal</u> cells in <u>culture</u>. GABA concentrations in brain and cultured glioma cells are comparable, whereas the neuroblastoma cells contain one-tenth as much. In general, the enzyme activities measured, <u>GABA transaminase</u>, <u>succinic semialdehyde dehydrogenase</u> and <u>glutamate dehydrogenase</u> are much lower in the cultured cells, and GABA transaminase is absent from the glial cells. The anticonvulsant drugs, aminooxyacetate (AOA) and dipropylacetate (DPA), elevated GABA concentrations in mouse brain and neuroblastoma cells. Only DPA affected the concentrations of GABA in glioma cells. The <u>convulsant drug</u>, isoniazid, depresses GABA levels in brain, had no effect in glioma cells and increased GABA in the neuroblastoma cells. </p>		

Project Description:

Objectives: To investigate the α -aminobutyric acid (GABA) shunt metabolites and enzymes in glioma and neuroblastoma cells in culture, and to study the effects of convulsant and anticonvulsant drugs.

Methods Employed: Cells are grown in a CO₂ incubator. Enzyme activities related to the GABA pathway are measured in homogenates of cells. Metabolites of the GABA shunt are measured in perchloric acid extracts of the cells. Both measurements employ sensitive enzymatic and/or radioisotope techniques.

Major Findings: The concentration of GABA in the neuroblastoma cell line is 10-fold greater than in the astrocytoma cells. The respective concentrations are 6.0 and 0.55 nmoles/mg protein, as compared with 11 nmoles/mg protein in mouse brain. Glutamate concentration in both cell lines was 90 nmoles/mg protein compared with 118 nmoles/mg protein in mouse brain. The concentrations of α -ketoglutarate were 0.81, 2.29, 0.97 nmoles/mg protein in mouse brain, astrocytoma cells, and neuroblastoma cells, respectively. GABA transaminase activity was at least 10-fold greater in neuroblastoma cells than in the astrocytoma cells, and there is some question that the enzyme may be lacking entirely in the glioma cells. Such a finding would explain the higher GABA concentrations in the astrocytoma cells, since further metabolism of GABA would not be possible: Glutamate dehydrogenase activity in the astrocytoma cells was half that in mouse brain, which is 1200 nmoles/mg protein/hr. The activity in the neuroblastoma cells was only a tenth of that in brain. The activity of succinic semialdehyde dehydrogenase was 145, 15, and 30 nmoles/mg protein/hr. in mouse brain, C-6 astrocytoma cells, and neuroblastoma cells, respectively. The effects of anticonvulsant and convulsant drugs were investigated. Aminooxyacetate, an anticonvulsant, elevated GABA concentrations in mouse brain and neuroblastoma cells, but not astrocytoma cells. Another anticonvulsant, dipropylacetate, increased GABA concentrations in mouse brain and in both cell lines. A convulsant drug, isoniazid, which depresses GABA levels in mouse brain, had no effect in the astrocytoma cells, and elevated GABA in the neuroblastoma cells. The relationship of the drug effects to the enzyme activities is under investigation.

Significance to Biomedical Research and the Program of the Institute: The mechanism of action of anticonvulsant and convulsant drugs has been the subject of extensive investigations. GABA, an inhibitory neurotransmitter, has been implicated as one possibility of drug action. When anticonvulsant drugs are administered to mice, the cerebellar levels of GABA increase, cyclic GMP decreases, and the threshold for seizures (electroconvulsive shock) is elevated. It is possible by using cells in culture to determine whether glioma and neuroblastoma cells are affected differently, and to isolate the site of action of the drugs.

Proposed Course of the Project: Further studies will be made to establish which drugs are acting on cells in culture and at which enzymic step. Cyclic GMP concentrations, which are altered in the brain of an intact animal, will also be analyzed.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01995-04 LNNS																				
PERIOD COVERED July 1, 1975 to June 30, 1976																						
TITLE OF PROJECT (80 characters or less) Morphological Studies of CNS Demyelination and Remyelination in Model Systems																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">H. deF. Webster</td> <td style="width: 35%;">Associate Chief</td> <td style="width: 15%;">LNNS NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>T. Tabira</td> <td>Visiting Fellow</td> <td>LNNS NINCDS</td> </tr> <tr> <td></td> <td>M. J. Cullen</td> <td>Staff Fellow</td> <td>LNNS NINCDS</td> </tr> <tr> <td></td> <td>P. J. Reier</td> <td>Assist. Prof. Anatomy</td> <td>U. of Md.</td> </tr> <tr> <td></td> <td>S. H. Wray</td> <td>Assist. Prof. Neurology</td> <td>Harvard Med. School</td> </tr> </table>			PI:	H. deF. Webster	Associate Chief	LNNS NINCDS	OTHER:	T. Tabira	Visiting Fellow	LNNS NINCDS		M. J. Cullen	Staff Fellow	LNNS NINCDS		P. J. Reier	Assist. Prof. Anatomy	U. of Md.		S. H. Wray	Assist. Prof. Neurology	Harvard Med. School
PI:	H. deF. Webster	Associate Chief	LNNS NINCDS																			
OTHER:	T. Tabira	Visiting Fellow	LNNS NINCDS																			
	M. J. Cullen	Staff Fellow	LNNS NINCDS																			
	P. J. Reier	Assist. Prof. Anatomy	U. of Md.																			
	S. H. Wray	Assist. Prof. Neurology	Harvard Med. School																			
COOPERATING UNITS (if any) Department of Anatomy, University of Maryland School of Medicine Department of Neurology, Harvard Medical School																						
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences																						
SECTION Section on Cellular Neuropathology																						
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014																						
TOTAL MANYEARS: <div style="text-align: center;">5.1</div>	PROFESSIONAL: <div style="text-align: center;">3.3</div>	OTHER: <div style="text-align: center;">1.8</div>																				
SUMMARY OF WORK (200 words or less - underline keywords) <p> The long range goal of this project is to use morphological methods such as <u>light and electron microscopy</u>, <u>freeze-fracture techniques</u>, and <u>electron dense tracers</u> to study mechanisms of CNS <u>demyelination</u> and <u>myelin formation</u> in an <u>in vivo</u> model of a myelinated CNS tract, the <u>optic nerves of Xeropus tadpoles</u>. Current studies include <u>myelin lesions</u> produced by <u>cerebrospinal fluid from multiple sclerosis patients</u>, <u>hexachlorophene intoxication</u>, and <u>low temperature</u>. </p>																						

Project Description:

Objectives: 1) Unconcentrated cerebrospinal fluid from more than 50% of patients with severe clinically active MS produces significantly elevated lesion counts in optic nerves of tadpoles 48 hrs. after it is injected perineurially. To investigate the mechanism of this myelinotoxic effect, the optic nerve lesions were studied at intervals after one or multiple injections of a positive CSF. 2) To study the effect of low temperature on CNS myelination in optic nerves of Xenopus tadpoles. 3) To determine whether hexachlorophene (HCP) has an effect on the size and distribution of intramembranous particles in CNS myelin.

Methods Employed: 1) Twelve μ l of unconcentrated CSF (either coded or myelinotoxic) or a saline control solution were injected around the right optic nerve of groups of stage 54 Xenopus tadpoles. At intervals thereafter (usually 48 hrs.), optic nerve whole mounts were prepared and randomized; then myelin lesions were counted by examining them with a differential-interference contrast microscope. 2) Groups of stage 54 Xenopus tadpoles were maintained at temperatures of 4°, 7°, 10°, and 15°C for 1-7 days. Some in the first 3 groups were sacrificed after 1, 3, and 7 days; the remainder recovered at room temperature for 1-2 weeks. After perfusion fixation, optic nerves were removed, postfixed, embedded and sectioned for electron microscopic study. 3) Groups of stage 54 Xenopus tadpoles were immersed in HCP, 0.2 μ gm/ml, for 1-10 days and freeze-fracture replicas of their optic nerves were prepared. Horseradish peroxidase was injected perineurially into tadpoles exposed to HCP for 7 days and their optic nerves were then prepared for electron microscopic examination.

Major Findings: 1) After a single injection of a myelinotoxic CSF, the most common optic nerve abnormality was the presence of small ovoids scattered at random along myelin internodes. They were most frequent in the middle segment of the nerve and in electron micrographs, the ovoids were found in areas of focal myelin breakdown. The number of myelin lesions reached a maximum two days after injection and decreased thereafter. If a CSF with myelinotoxic activity was diluted or heated for 30 minutes at 60°C before it was injected, the number of myelin lesions was similar to that found in controls. After multiple injections of a myelinotoxic CSF, the optic nerves were much more abnormal. Breakdown of myelin sheaths was more extensive and some demyelinated axons were observed. 2) When Xenopus tadpoles were maintained at 4-10°C for 3-7 days, there were abnormalities in myelinated axons and glial cells. Altered oligodendrocyte processes were associated with axons containing fewer than normal numbers of microtubules. Inner tongue processes of these myelinated fibers were frequently enlarged and contained membranous honeycomb structures. Changes in outer tongue processes were similar but less frequent. In tadpoles maintained at 7°, the optic nerves were transected; the above changes were not observed distally nor were they seen in optic nerves of tadpoles injected with colchicine or vinblastine. 3) In optic nerves from tadpoles immersed in HCP, the inner half of myelin membranes adjacent to intramyelinic vacuoles contained numerous particles-whose size and random distribution were similar to that seen in normal

sheaths. Often, however, there were focal, particle-free elevations on membrane surfaces bounding vacuoles; such were not found in normal myelin. These particle-free regions frequently projected into the myelin vacuoles and were similar to the multivesiculated membrane blebs observed in thin sections. Strands of particles, corresponding to light junctions commonly seen in normal myelin sheaths, were also present on inner membrane surfaces of vacuolated sheaths. These particles did not appear to be altered by HCP exposure. When a macromolecular tracer, horseradish peroxidase, was injected perineurally in tadpoles exposed to HCP, it readily penetrated the optic nerve parenchyma; none was found in the myelin vacuoles.

Significance to Biomedical Research and the Program of the Institute:

1) The results of our double blind tests strongly suggest that the CSF of many patients with severe clinically active multiple sclerosis contains a heat labile factor that has a concentration dependent toxic effect on the CNS myelin sheaths in tadpole optic nerves. Fractions of positive CSFs are currently being prepared and tested in order to characterize the nature of the CSF myelinotoxic factor. The results also have shown our double blind CSF test is useful for investigating mechanisms of myelin breakdown in multiple sclerosis and other demyelinating diseases. Only 0.5 ml. of unconcentrated CSF is needed and the result is known in 5 days. 2) Our data show that maintenance of tadpoles at low temperature produces distinctive changes in oligodendrocytes that are forming myelin. These alterations are not seen during Wallerian degeneration nor are they directly related to the dissociation of microtubules. 3) The above results show that the massive vacuolation and splitting of CNS myelin sheaths produced by HCP is not associated with diffuse changes in the number, size, and distribution of intramembranous particles. Also, the structure of tight junctions in the sheath remains normal and penetration of macromolecular tracers into the vacuolar myelin lesions is prevented.

Proposed Course of the Project: To be continued. The above results have been presented at the Annual Meetings of the American Association of Anatomists and the American Association of Neuropathologists.

Publications: Tabira, T., Webster, H. deF., and Wray, S. H.: Demyelinating activity of cerebrospinal fluid from multiple sclerosis patients tested in a new model system, the optic nerves of Xenopus tadpoles. Trans. ANA 100: 103-106, 1975.

Reier, P. J., Tabira, T., and Webster, H. deF.: The penetration of fluorescent and electron dense tracers into Xenopus tadpole optic nerves following perineural injection. Brain Res. 102: 229-244, 1976.

Tabira, T., Webster, H. deF., and Wray, S. H.: In vivo test for myelinotoxicity of cerebrospinal fluid. Brain Res., 1976 (in press).

Tabira, T., Webster, H. deF., and Wray, S. H.: Multiple sclerosis: Cerebrospinal fluid produces myelin lesions in tadpole optic nerves. New Eng. J. Med., 1976 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01996-04 LNNS
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Membrane Structure in CNS Tissue and Subcellular Brain Fractions		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	H. deF. Webster P. J. Reier R. H. Quarles J.-M. Matthieu	Associate Chief Assist. Prof. Anatomy Research Chemist Assist. Prof. Pediatrics LNNS NINCDS U. of Md. DMN NINCDS U. of Lausanne
COOPERATING UNITS (if any) Department of Anatomy, University of Maryland School of Medicine Developmental and Metabolic Neurology Branch, NINCDS University of Lausanne School of Medicine, Lausanne, Switzerland		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cellular Neuropathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.35	OTHER: 0.05
SUMMARY OF WORK (200 words or less - underline keywords) The long range goal of this project is to use <u>freeze fracture techniques</u> and <u>electron microscopy</u> to study the structure of <u>myelin</u> and <u>cell membranes</u> in <u>CNS tissue</u> and in <u>subcellular fractions</u> . Current experiments include examination of <u>optic nerves</u> from the mouse mutant, <u>Jimmy</u> , and the study of <u>CNS myelin fractions</u> prepared from <u>Xenopus</u> brains and spinal cords.		

Project Description:

Objectives: 1) To study the distribution of axon membrane particles in freeze-fracture preparations of optic nerves from neonatal Jimpy and control mice and also to determine the diameters of these axons and their relationships with glial cell processes. 2) To isolate myelin from Xenopus frogs and correlate the electron microscopic appearance of myelin fractions with their protein and lipid composition (Dr. Quarles, Z01 NS 01808-06 DMN).

Methods: 1) Freeze-fracture replicas of lightly fixed optic nerves from 9 and 18 d. Jimpy and control mice were prepared and studied with the electron microscope. Other blocks of the same optic nerves were postfixed, embedded, and sectioned for electron microscopic examination. 2) Brains and spinal cords of Xenopus were used to prepare CNS myelin fractions which were fixed, dehydrated, embedded, and sectioned for electron microscopic examination.

Major Findings: 1) In electron micrographs of longitudinally fractured optic nerves, there were fewer particles on cytoplasmic faces of Jimpy axon membranes than on control axolemmas at 9 and 18 d. of age. Measurements of axon diameters at these ages showed that in Jimpy, growth of axons was retarded and that smaller axons were more severely affected. Also, at 9 d. of age, more than 90% of the axons in control and Jimpy optic nerves had other axons as neighbors. The percentage of axons that were surrounded by glial processes was higher in Jimpy nerves but many of these processes belonged to astrocytes instead of oligodendrocytes. At 9 d., about 2% of the axons in Jimpy and control nerves were surrounded by loose and compact myelin spirals. By 18 d., however, compact myelin sheaths surrounded almost 50% of control axons but only 1% in Jimpy, the same percentage found at 9 d. 2) Multilayered membranous whorls with the characteristic lamellar appearance and periodicity of myelin were present in fractions isolated from Xenopus brain and spinal cord. They did not differ significantly from those seen in myelin fractions isolated from rat, mouse, and bovine CNS.

Significance to Biomedical Research and the Program of the Institute:

1) In the mouse mutant, Jimpy, there is a severe defect in CNS myelination. The major abnormalities are found in oligodendrocytes; there are alterations also in the few myelin sheaths that are formed. The cellular mechanisms responsible for the defect in CNS myelination are still poorly understood and the above observations are among the first to suggest that it may be associated with abnormal axonal development. However, additional data, including studies of other axonal populations, will be needed to assess the significance of our results. 2) The characterization of adult and immature Xenopus myelin has become an important priority because it has recently been shown in CN, LNNS, NINCDS, that the optic nerves of Xenopus tadpoles are a useful in vivo model for studies of experimental and human demyelinating diseases.

Proposed Course of the Project: To be continued. The above findings have been presented at the Annual Meeting of the American Association of Neuropathologists.

Publications: Reier, P. J., Matthieu, J.-M., and Zimmerman, A. W.: Myelin deficiency in hereditary pituitary dwarfism: A biochemical and morphological study. J. Neuropath. Exp. Neurol. 34: 465-477, 1975.

Zimmerman, A. W., Quarles, R. H., Webster, H. deF., Matthieu, J.-M., and Brady, R. O.: Characterization and protein analysis of myelin subfractions in rat brain: Developmental and regional comparisons. J. Neurochem. 25: 749-758, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02105-03 LNNS
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Behavior of the Blood-Brain Barrier and the Development of Edema in Cerebral Ischemia		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: U. Ito OTHER: K. G. Go M. Spatz I. Klatzo	Visiting Fellow Assoc. Prof. Neurosurgery Head, Section on Neurocytobiol. Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS Univ. Groningen LNNS NINCDS LNNS NINCDS
COOPERATING UNITS (if any) University of Groningen Groningen, The Netherlands		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 2.3	PROFESSIONAL: 1.7	OTHER: 0.6
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> The incidence of the <u>blood-brain barrier (BBB) damage</u>, evident by blue staining of the brain tissue, was related to the duration of ischemia and to the duration of release following carotid occlusion. Thus, e.g., in gerbils subjected to 6 hours ischemia, all animals showed the BBB damage 1 hour after release of occlusion. On the other hand, gerbils with 1 hour left carotid occlusion showed 100% incidence of the BBB damage only 20 hours following release of carotid clipping. Raising the <u>systemic arterial blood pressure</u> accelerated significantly the BBB damage; e.g., a 100% incidence of the BBB damage was evident in gerbils with 147.8 mean arterial blood pressure (MABP) within 15 minutes after release, whereas in animals with the normal MABP (72.4), such incidence was present only after 5 hours of release of carotid occlusion. The histological changes characteristic of ischemic damage clearly preceded the BBB injury. </p>		

Project Description:

Objectives: One of the complications of cerebral ischemia is the development of brain edema. Two main types of brain edema are the cytotoxic and vasogenic varieties which can be differentiated on the basis of the behavior of the blood-brain barrier (BBB). The objective of this study has been to elucidate the nature of ischemic brain edema by studying in a model of experimental cerebral ischemia the behavior of the BBB and the abnormal increase in brain tissue fluid, the essential criterion of any type of brain edema.

Methods Employed: Mongolian gerbils (*Meriones unguiculatus*) in which, due to anatomical irregularities of the circulus of Willis, an occlusion of the common carotid artery on the neck produces in about 30% of animals an ischemic injury in the ipsilateral cerebral hemisphere, were used as an experimental model for cerebral ischemia. In this project, the left common carotid artery was clipped on the neck for various periods of time. For evaluation of the BBB, Evans blue dye was injected intravenously as a tracer. The systemic arterial blood pressure was monitored by intrafemoral catheter connected with a transducer. Abnormal increase of the brain tissue fluid was estimated by wet/dry weight ratios. After decapitation, the brains were taken out and the separated hemispheres were weighed (fresh weight). After drying at 98°C for six days, the samples were weighed again (dry weight). Histological changes were evaluated in brain tissue fixed by paraformaldehyde perfusion, embedded in paraffin and stained with hematoxylin eosin and cresyl violet.

Major Findings: The estimation of wet/dry weight ratios revealed a progressive accumulation of the fluid in relation to the duration of carotid occlusion. Definite evidence of brain edema was apparent in animals sacrificed after 3 hours of carotid clipping which showed 7.34 ± 1.01 mean swelling percentage in the left hemisphere. In animals with 18 hours occlusion, the swelling of the infarcted hemisphere amounted to 22%. The incidence of the BBB damage, evident by blue staining of the brain tissue, was related to the duration of ischemia and to the duration of release following carotid occlusion. Thus, e.g., in gerbils subjected to 6 hours ischemia, all animals showed the BBB damage 1 hour after release of occlusion. On the other hand, gerbils with 1 hour left carotid occlusion showed 100% incidence of the BBB damage only 20 hours following release of carotid clipping. Raising the systemic arterial blood pressure accelerated significantly the BBB damage; e.g., a 100% incidence of the BBB damage was evident in gerbils with 147.8 mean arterial blood pressure (MABP) within 15 minutes after release, whereas in animals with the normal MABP (72.4), such incidence was present only after 5 hours of release of carotid occlusion. The histological changes characteristic of ischemic damage clearly preceded the BBB injury. They were first recognizable in animals sacrificed 30 minutes after carotid occlusion. They were progressively increasing during the period of release of carotid occlusion. This was in agreement with the observation that the histological picture of ischemic damage was similar in intensity in gerbils sacrificed immediately 6 hours after ischemia with that in animals with 1 hour occlusion and sacrificed 20 hours after release of the clipping.

Significance to Biomedical Research and the Program of the Institute:

The elucidation of the nature of ischemic brain edema is potentially of great value for the clinical management of stroke patients. The studies described above indicate that following ischemia there is a development of brain edema which is primarily of cytotoxic type. Only in later stages when there is damage to cerebral blood vessels and their increased permeability to BBB tracers, a leakage of serum components is adding a vasogenic component to overall picture of brain edema. These findings might be of importance for designing proper treatment of patients suffering from cerebral ischemia.

Proposed Course of the Project: This project has been completed.

Publications: Ito, U., Go, K. G., Walker, J. T., Jr., Spatz, M., and Klatzo, I.: Experimental cerebral ischemia in Mongolian gerbils. III. Behaviour of the blood-brain barrier. Acta Neuropath. 34: 1-6, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02150-02 LNNS
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Electronmicroscopic Studies of the Hippocampus in Gerbils Subjected to Cerebral Ischemia		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: J. J. Bubis OTHER: T. Fujimoto B. J. Mrsulja I. Klatzo	Visiting Scientist Visiting Fellow Visiting Fellow Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS LNNS NINCDS LNNS NINCDS LNNS NINCDS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 1.35	PROFESSIONAL: 1.25	OTHER: .10
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> Light microscopic observations of the resin embedded tissue showed the affected neurons as rounded cells with the characteristic eccentric nucleus, central cytoplasmic dark granules and some vacuoles. Under the electron microscope, the central part of the cytoplasm was devoid of endoplasmic reticulum and it was filled with <u>mitochondria of bizarre shape</u> and of different sizes, with numerous <u>lysosome-like structures</u>, with several vacuoles and with small (60-80 nm) electron-dense inclusions of ragged contour and not membrane bound; these inclusions may represent glycogen deposits. The <u>Golgi apparatus</u> was either absent or was seen in the form of irregularly arranged cisternae or of a group of vesicles. <u>Thiamine pyrophosphatase reaction</u> was seen in some short cisternae or vesicles. <u>Acid phosphatase activity</u> was present in many of the lysosome-like structures suggesting the formation of secondary lysosomes and autophagosomes. </p>		

Project Description:

Objectives: In a previous study (Ito, U., Spatz, M., Walker, J. T., Jr., and Klatzo, I.: Experimental cerebral ischemia in Mongolian gerbils. I. Light microscopic observations. Acta Neuropath. 32: 209-223, 1975) a marked neuronal reaction in H2 sector of Ammon's horn was observed in gerbils subjected to 15 minutes of common carotid artery clipping and 20 hours release. This lesion was characterized by prominent cells with eccentric nuclei, central cytoplasmic eosinophilia and either complete absence or peripheral cytoplasmic presence of Nissl bodies. These light microscopic changes are morphologically similar to some cases of neuronal chromatolysis. The present study was designated to investigate the morphologic and histoenzymatic changes of this ischemic lesion by electronmicroscopy.

Methods Employed: Gerbils were subjected to unilateral or bilateral clamping of the carotid arteries for periods ranging from 7 to 15 minutes. Twenty hours after release of the clamp, the animals were perfused through the heart with 15 ml of balanced salt solution followed by 1% paraformaldehyde-1.25% glutaraldehyde solution, and then by a 4% paraformaldehyde-5% glutaraldehyde fixing solution. Three hours afterwards, the brain was removed, the selected areas of hippocampus were cut and processed for Araldite or Epon embedding. For histochemistry only the 1% paraformaldehyde-1.25% glutaraldehyde fixing solution was used, the brain was removed 30 minutes after perfusion, and 50 μ thick sections from selected hippocampal regions were incubated for acid phosphatase, thiamine pyrophosphatase and endogenous peroxidase and then processed for electronmicroscopy.

Major Findings: Light microscopic observations of the resin embedded tissue showed the affected neurons as rounded cells with the characteristic eccentric nucleus, central cytoplasmic dark granules and some vacuoles.

Under the electron microscope, the eccentric nucleus had a kidney-like shape with its concavity facing the center of the cell. Irregular and short cisternae of the rough endoplasmic reticulum and also membrane-free ribosomes were seen at the periphery of the cell. The central part of the cytoplasm was devoid of endoplasmic reticulum and it was filled with mitochondria of bizarre shape and of different sizes, with numerous lysosome-like structures, with several vacuoles and with small (60-80 nm) electron-dense inclusions of ragged contour and not membrane bound; these inclusions may represent glycogen deposits. The Golgi apparatus was either absent or was seen in the form of irregularly arranged cisternae or of a group of vesicles. Thiamine pyrophosphatase reaction was seen in some short cisternae or vesicles.

Acid phosphatase activity was present in many of the lysosome-like structures suggesting the formation of secondary lysosomes and autophasosomes. No normal GERL (Novikoff) was seen in the affected neurons.

Significance to Biomedical Research and the Program of the Institute: The structural and functional elucidation of cerebral lesions caused by ischemia of short duration is of great clinical importance for the basic

understanding of cerebrovascular disease process as well as its prevention and possible therapy.

Proposed Course of the Project: This project has been completed and the manuscript of the paper has been sent for publication in Acta Neuropath.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02174-02 LNNS
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Histochemical Investigation of the Mongolian Gerbil's Brain During Unilateral Ischemia		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: B. J. Mrsulja OTHER: M. Spatz I. Klatzo	Visiting Fellow Head, Section on Neurocytobiol. Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS LNNS NINCDS LNNS NINCDS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: .85	PROFESSIONAL: .75	OTHER: .10
SUMMARY OF WORK (200 words or less - underline keywords) <p> The first detectable changes in the activities of all <u>dehydrogenases</u> was seen as a slight decrease in the staining intensity of ischemic hemisphere in animals subjected to 2 hours ischemia. The greatest diminution of the demonstrable enzymatic staining product was seen after 9 hours of ischemia. The slight decrease of non-specific <u>acid monophosphatase</u> activity was also not seen prior to 2 hours ischemia. In later stages of ischemia, a loss of enzymatic activity was seen in the severely damaged region, while the surrounding areas showed an increased activity of the acid phosphatase. No significant change in the activity of alkaline phosphatase was demonstrated in ischemic regions at any time. Animals killed within 1 hour of ischemia showed a diminution of <u>phosphorylase</u> activity prominent on the ischemic side. By 6 and 9 hours of ischemia, phosphorylase activity was completely suppressed. Necrotic areas revealed markedly diminished or complete loss of total phosphorylase activity, while the rest of the hemisphere exhibited hyperactivity of the same enzyme and increased <u>glycogen</u> content, especially close to the necrotic areas. </p>		

Project Description:

Objectives: The Mongolian gerbils possess a high susceptibility for the development of cerebral infarction following unilateral ligation of the common carotid artery due to frequent absence of arterial communication between the cerebral and vertebral system. The contralateral hemisphere then serves as a control (Kahn, K., *Neurol.* 22: 510-515, 1972). The purpose of the present investigation was to study ischemic effect on the various enzymes activities in the brain histochemically.

Methods Employed: The histochemical investigation was performed on several groups of Mongolian gerbils which were subjected to unilateral ischemia of 30 and 60 minutes, 2, 3, 6 and 9 hours. Fresh frozen sections were used for the histochemical enzyme evaluation of the ischemic and control hemispheres. The following enzymes were investigated: respiratory enzymes succinic (SDH), lactic (L-DH), glutamic (G-DH), glucose-6-phosphate (G-6PDH); dehydrogenases were demonstrated by Nitro BT-methods. Cytochrome oxidase (COX) activity was studied according to Burstone's p-aminodiphenylamine method. Sections heated at 60°C prior to incubation served as controls. L-leucyl- β -naphthylamide was used as a substrate according to the Gomori's modified method by Burstone and Fold. Alkaline and acid non-specific phosphatase activities were assessed by Burstone's naphthol AS-phosphatase methods using naphthol AS-TR as a substrate for both enzymes but with different pH of the incubating medium. Total glycogen phosphorylase activity was demonstrated by Takeuchi and Kuriaki. The brain sections were incubated at 37°C in Navikoff's and Goldfisher's (1961) medium for thiamine pyrophosphatase (TPP-ase). For evaluation of glycogen, the brain tissue was fixed by perfusion with paraformaldehyde embedded in paraffin and stained according to the cold Schiff's method, preceded by 1 hour of incubation in 5% Dimedon (5,5'-Dimethyl-1,3-cyclohexadion) solution (Bulmer, D., *Stain Tech.* 34: 95, 1959).

Major Findings: The first detectable changes in the activities of all dehydrogenases was seen as a slight decrease in the staining intensity of ischemic hemisphere in animals subjected to 2 hours ischemia. The loss of the dehydrogenase activities was parallel with the duration of ischemia. The greatest diminution of the demonstrable enzymatic staining product was seen after 9 hours of ischemia. The slight decrease of non-specific acid monophosphatase activity was also not seen prior to 2 hours ischemia. It manifested itself by slight decrease of stainable neuronal cytoplasm granule in H2 and H3 sector of the Ammon's horn and thalamus. However, at the periphery of the ischemic lesion (in the thalamo-hypothalamic region) a higher more diffuse (nongranular) cytoplasmic activity of the acid phosphatase was seen than on the contralateral control side. In later stages of ischemia, a loss of enzymatic activity was seen in the severely damaged region while the surrounding areas showed an increased activity of the acid phosphatase. No significant change in the activity of alkaline phosphatase was demonstrated in ischemic regions at any time. Cytochemical studies of the Golgi apparatus revealed no abnormalities earlier than after 3 hours. At this stage of ischemia, the cortex, hippocampus and thalamus showed very

slight decrease of the staining intensity. In many of these places, the neurons were completely devoid of enzyme activity. In addition to this, there was an unusual increase in the number of neurons with diffuse or granular cytoplasmic and nuclear staining. In zones of advanced necrosis, diminished staining of the blood vessels and glia were seen in the TPP-ase preparations. Total phosphorylase was the only one enzyme which was changed after 1 hour of ischemia. Animals killed within 1 hour of ischemia showed a diminution of phosphorylase activity prominent on the ischemic side. By 6 and 9 hours of ischemia, phosphorylase activity was completely suppressed. Necrotic areas revealed markedly diminished or complete loss of total phosphorylase activity, while the rest of the hemisphere exhibited hyperactivity of the same enzyme and increased glycogen content, especially close to the necrotic areas. Cytologically, the increased glycogen and enzymatic activity were situated mainly within the astrocytes and in the neuropil.

Significance to Biomedical Research and the Program of the Institute:

The histochemical evaluation of several groups of enzymes after various periods of brain ischemia will be helpful in assessing the extent of ischemic effect in cerebral metabolism. Such studies are very important for the understanding of the ischemic process: development, arrest, and possible prevention.

Proposed Course of the Project: This project has been completed and the results are being described and prepared for publication.

Publications: None



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02175-02 LNNS
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Histochemical Observation on Mongolian Gerbils' Brains During and After Regional Ischemia		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: B. J. Mrsulja OTHER: I. Klatzo M. Spatz U. Ito	Visiting Fellow Chief, Lab. Neuropath. Neuroanat. Sci. Head, Section on Neurocytobiol. Visiting Fellow	LNNS NINCDS LNNS NINCDS LNNS NINCDS LNNS NINCDS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: .85	PROFESSIONAL: .75	OTHER: .10
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> The <u>histochemical</u> regional ischemic <u>changes</u> have been directly related to the duration of the blood supply restriction to the brain and the following period of reestablished circulation. At the end of ischemic insult and after 1 hour of common carotid artery clip release, no differences in enzymatic activities were seen between ischemic and non-ischemic Ammon's horn. Twenty hours after restoration of cerebral circulation, the <u>oxidative mitochondrial enzymes S-DH, L-DH, G-DH, G-6-DH and C-OH</u> were markedly increased in the pyramidal cells of sabiculum and H3 sector of the hippocampus. The proteolytic enzyme <u>aminopeptidase</u> and the Golgi's marker <u>TPP-ase</u> were also increased in the same part of Ammon's horn. </p>		

Project Description:

Objectives: In previous studies we have observed a "maturation phenomena" of cerebral lesions in unilateral ischemia of gerbils. The rate of "maturation" of the ischemic lesions was directly related to the intensity (duration) of the ischemic insult (Ito, U., Spatz, M., Walker, J. T., Jr. and Klatzo, I.: Experimental cerebral ischemia in Mongolian gerbils. I. Light microscopic observations. Acta Neuropath. 32: 209-223, 1975). The investigation reported here was designated to evaluate and possibly elucidate the mechanism responsible for this phenomena.

Methods Employed: Regional cerebral ischemia was produced in Mongolian gerbils by clipping of the common carotid artery (unilateral or bilateral) for various periods of time. Animals with neurological signs of infarction were sacrificed either immediately after the end of the ischemic period (7.5, 15, 60 and 180 minutes), or after 1, 5, 20 hours, 1 and 4 weeks of recirculation. The following enzymes were investigated: respiratory enzymes succinic (SDH), lactic (LDH), glutamic (G-DH), glucose-6-phosphate (G-6PDH); dehydrogenases were demonstrated by Nitro BT-methods. Cytochrome oxidase (COX) activity was studied according to Burstone's p-aminodiphenyl-anine method. Sections heated to 60°C prior to incubation served as controls. L-leucyl- β -naphthylamide was used as a substrate according to the Gomori's modified method by Burstone and Fold. Alkaline and acid non-specific phosphatase activities were assessed by Burstone's naphthol AS-phosphate methods, using naphthol AS-TR as a substrate for both enzymes, but with different pH of the incubating medium. Total glycogen-phosphorylase activity was demonstrated by Takeuchi and Kuriari. The brain sections were incubated at 37°C in Navikoff's and Goldfisher's (1961) medium for thiamine pyrophosphatase (TPP-ase). For glycogen demonstration the brain tissue was fixed by perfusion with paraformaldehyde embedded in paraffin and stained according to the cold Schiff's method, preceded by 1 hour of incubation in 5% Dimedon (5,5-Dimethyl-1,3-cyclohexadion) solution (Bulmer, D., Stain Tech. 34: 95, 1959).

Major Findings: The histochemical regional ischemic changes have been directly related to the duration of the blood supply restriction to the brain and the following period of reestablished circulation. However, individual variation in the activity of all enzymes examined were also found in the group of animals subjected to the same duration of experimental ischemia. The experimental animals were divided into two distinct groups according to the histochemical enzymatic observations: (1) short period of ischemia (7.5 and 15 minutes) with various recovery periods: At the end of ischemic insult and after 1 hour of common carotid artery clip release, no differences in enzymatic activities were seen between ischemic and non-ischemic Ammon's horn. Twenty hours after restoration of cerebral circulation, the oxidative mitochondrial enzymes S-DH, L-DH, G-DH, G-6-DH and C-OH were markedly increased in the pyramidal cells of sabiculum and H3 sector of the hippocampus. The proteolytic enzyme aminopeptidase and the Golgi's marker TPP-ase were also increased in the same part of Ammon's horn. Furthermore, the activity of acid phosphatase was seen as an increased diffuse and homogeneous

reaction in the neuronal cytoplasm of the affected ischemic side, as compared to the cytoplasmic granular reaction on the control side. One week after the ischemic insult all of the above mentioned enzymes were increased as compared to controls, but to a lesser degree than after 20 hours of clip release. In addition, the H2 sector showed focally increased activity of all above mentioned enzymes. (2) Long period of ischemia (1 and 3 hours) with various recovery periods: The activity of all investigated enzymes in the brain was decreased at each of the examined periods of re-established cerebral circulation. The loss of activity was progressive and directly related to the duration of the restored circulation. The difference in the intensity of the observed activity between the ischemic and non-ischemic hemisphere was slight at 1 hour but extremely marked at 1 week after the ischemic insult. However, the group of animals subjected to 1 hour carotid artery occlusion and 1 week release show a characteristic exception to this rule; namely, an increased activity of lysosomal and proteolytic enzymes.

Significance to Biomedical Research and the Program of the Institute:

Upon completion of this study, it will be possible to ascertain the relationship of the intensity (duration) of ischemic insult to the recovery of cerebral lesions. The basic evaluation of cerebrocellular recovery potential is of great prognostic and therapeutic importance, clinically.

Proposed Course of the Project: This project has been completed and the manuscript pertaining to this work is being prepared for publication.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZQ1 NS Q2176-Q2 LNNS
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Action of Cerebral Ischemia on Decreased Levels of Catechol and Indol Amine Metabolites		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: B. B. Mrsulja OTHER: B. J. Mrsulja M. Spatz I. Klatzo	Visiting Scientist Visiting Fellow Head, Section on Neurocytobiol. Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS LNNS NINCDS LNNS NINCDS LNNS NINCDS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.08	PROFESSIONAL: .08	OTHER: 0
SUMMARY OF WORK (200 words or less - underline keywords) <p> <u>Cerebral ischemia</u> of 30 minutes duration significantly increases the level of HVA, while it has no influence on MOPEG-SO₄ and 5-HIAA levels. MOPEG-SO₄ level was not changed 1 hour after onset of ischemia, while that of 5-HIAA was increased. However, MOPEG-SO₄ level was increased after 2 hours of ischemia. It took about 2 hours for catecholamine metabolites to reach the highest values, and 1 hour for 5-HIAA. Thereafter, these levels remained unchanged. In gerbils' brains, inhibitor of monoamine oxidase, <u>pargyline</u>, significantly decreased the levels of HVA, MOPEG-SO₄ and 5-HIAA. This decrease could be prevented by: (1) blockage of active out-transport of metabolites from the brain of CSF by means of <u>probenecid</u> or (2) by the exposure of the animals to ischemia. It appears that ischemia has the same influence on pargyline-treated animals as probenecid. </p>		

Project Description:

Objectives: The catechol and indol amines are thought to play an important role in cerebral function and their relationship to neuro-pathology is also pointed out. In cerebral ischemia we found the changes of dopamine, norepinephrine and serotonin and here we are reporting about the changes of their main metabolites, homovanillic acid (HVA), 3-methoxy-4-hydroxy-phenyl-ethylglycol-sulphate (MOPEG-SO₄) and 5-hydroxy-indolacetic acid (5-HIAA), respectively.

Methods Employed: Experiments were conducted on Mongolian gerbils. Unilateral cerebral ischemia was produced by ligation of one common carotid artery of the neck. Following ligature, the animals with neurological signs of cerebral infarction were sacrificed 30 minutes, 1, 2, 3 and 6 hours after onset of ischemia. Also, gerbils received pargyline (75 mg/kg) 30 minutes prior to treatment with probenecid (200 mg/kg) or production of ischemia. In this experiment, animals were sacrificed 2 hours thereafter. In all animals in ischemic and control hemisphere, concentrations of HVA, MOPEG-SO₄ and 5-HIAA were analyzed spectrophotofluorometrically.

Major Findings: Cerebral ischemia of 30 minutes duration significantly increases the level of HVA, while it has no influence on MOPEG-SO₄ and 5-HIAA levels. MOPEG-SO₄ level was not changed 1 hour after onset of ischemic, while that of 5-HIAA was increased. However, MOPEG-SO₄ level was increased after 2 hours of ischemia. It took about 2 hours for catecholamine metabolites to reach the highest values, and 1 hour for 5-HIAA. Thereafter, these levels remained unchanged. In gerbils' brains, inhibitor of mono-amine oxidase, pargyline, significantly decreased the levels of HVA, MOPEG-SO₄ and 5-HIAA. This decrease could be prevented by: (1) blockage of active out-transport of metabolites from the brain of CSF by means of probenecid, or (2) by the exposure of the animals to ischemia. It appears that ischemia has the same influence on pargyline-treated animals as probenecid.

Significance to Biomedical Research and the Program of the Institute: In addition to the well known increase of lactate in ischemia which influences the pH of nervous tissue, retention of organic acids, metabolites of catechol and indol amines might also be a factor involved in producing tissue acidosis. This retention in ischemia is probably due to the altered out-transport of HVA, MOPEG-SO₄ and 5-HIAA from the tissue and CSF.

Proposed Course of the Project: This project has been completed and the resulting paper has been sent for publication.

Publications: None

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Steady State Kinetics In Catecholamine and Serotonin Turnover in Cerebral Ischemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. B. Mrsulja	Visiting Scientist	LNNS NINCDS
OTHER:	B. J. Mrsulja	Visiting Fellow	LNNS NINCDS
	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat.	LNNS NINCDS
		Sci.	

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

.08

PROFESSIONAL:

.08

OTHER:

0

SUMMARY OF WORK (200 words or less - underline keywords)

The cerebral ischemia in gerbils is accompanied by: (1) decreased rate of DA, NOR and 5-HT synthesis in cerebral cortex as indicated by: (a) decreased accumulation of NOR and 5-HT after inhibition of monoamine oxidase by means of pargyline, and (b) further reduction of DA, NOR and 5-HT after inhibition of tyrosine hydroxylase and tryptophan hydroxylase by means of AMPT and PCPA, respectively. (2) An altered active out-transport of HVA, MOPEG-SO₄ and 5-HIAA from the tissue or CSF as indicated by: (a) increased accumulation of HVA, MOPEG-SO₄ and 5-HIAA in ischemia following the inhibition of active transport by means of probenecid, and (b) accumulation of HVA and MOPEG-SO₄ in ischemia following inhibition of catecholamine synthesis by means of AMPT and accumulation of 5-HIAA after inhibition of 5-HT synthesis by means of PCPA.

Project Description:

Objectives: Gerbils exhibiting cerebral infarction showed in cerebral cortex decreased levels of dopamine (DA), norepinephrine (NOR), and serotonin (5-HT) and increased levels of homovanillic acid (HVA), 3-methoxy-4-hydroxy-phenylethylglycol-sulphate (MOPEG-SO₄) and 5-hydroxy-indolacetic acid (5-HIAA) metabolites of monoamines, respectively. In order to clarify behavior or biogenic amines and their main metabolites in ischemia, we applied pharmacologic measurement of amine turnover and steady-state kinetics studies.

Methods Employed: All analyses were conducted on Mongolian gerbils showing the neurological signs of cerebral infarction. Two to four hours prior to the common carotic artery occlusion, the following modulators of biogenic amine metabolism were injected i.p.: pargyline (75 mg/kg), alpha-methyl-p-tyrosine (AMPT, 400 mg/kg), p-chlorophenylalanine (PCPA, 500 mg/kg), probenecid (200 mg/kg), pyrogallol (200 mg/kg), and 2-3 hours after occlusion the levels of DA, NOR, 5-HT, HVA, MOPEG-SO₄ and 5-HIAA were measured fluorometrically. Steady-state kinetics study was applied to calculate the turnover rate and turnover time of biogenic amines in ischemia.

Major Findings: Our results suggest that the cerebral ischemia in gerbils is accompanied by: (1) decreased rate of DA, NOR and 5-HT synthesis in cerebral cortex as indicated by: (a) decreased accumulation of NOR and 5-HT after inhibition of monoamine oxidase by means of pargyline, and (b) further reduction of DA, NOR, and 5-HT after inhibition of tyrosine hydroxylase and tryptophan hydroxylase by means of AMPT and PCPA, respectively. (2) An altered active out-transport of HVA, MOPEG-SO₄ and 5-HIAA from the tissue or CSF as indicated by: (a) increased accumulation of HVA, MOPEG-SO₄ and 5-HIAA in ischemia following the inhibition of active transport by means of probenecid, and (b) accumulation of HVA and MOPEG-SO₄ in ischemia following inhibition of catecholamine synthesis by means of AMPT and accumulation of 5-HIAA after inhibition of 5-HT synthesis by means of PCPA. (3) Possible potentiated O-methylation as indicated by increased levels of DA and NOR following inhibition of catechol-O-methyl-transferase by means of pyrogallol.

Significance to Biomedical Research and the Program of the Institute: Our results suggest synaptosomal release and probably depletion of biogenic amines during ischemia, inhibition of synthesis and degradation of these monoamines and alteration of active transport of their main metabolites from the brain tissue. Such behavior of catechol and indol amines and their metabolites in ischemia might be responsible or might cause further ischemic injury in surrounding brain tissue primarily not affected. At the same time, data implicates the possibility of using drugs which can modify the level or action of brain monoamines and at least the extension of ischemic brain damage.

Proposed Course of the Project: This project has been completed.

Publications: Mrsulja, B. B., Mrsulja, B. J., Spatz, M. and Klatzo, I.:
Action of cerebral ischemia on decreased levels of 3-methoxy-
4-hydroxy-phenylethylglycol sulfate, homovanillic acid and
5-hydroxy-indoleacetic acid produced by pargyline. Brain
Res. 98: 388-393, 1975.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02178-02 LNNS
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Behavior of Biogenic Amines In and Following Cerebral Ischemia		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: B. B. Mrsulja OTHER: B. J. Mrsulja M. Spatz I. Klatzo	Visiting Scientist Visiting Fellow Head, Section on Neurocytobiol. Chief. Lab. Neuropath Neuroanat. Sci.	LNNS NINCDS LNNS NINCDS LNNS NINCDS LNNS NINCDS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: .08	PROFESSIONAL: .08	OTHER: 0
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>One hour after onset of ischemia, <u>5-HT</u> level was significantly increased in ischemic hemispheres, and was not further changed up to 6 hours duration of ischemia. The levels of <u>DA</u> and <u>NOR</u> were reduced 1 hour after the occlusion was established and the reduction of these concentrations were proportional to the duration of ischemia. In recovery after ischemia, one hour and 15 minute occlusion, as well as 1 hour after 1 hour occlusion, 5-HT level was significantly higher than it was during ischemia, while in gerbils which were ischemic for longer periods, 5-HT levels were significantly lower than in ischemia. Twenty hours after the clip was removed, 5-HT level in animals which were ischemic only for 15 minutes reached the peak and was higher than 1 hour after the clip was removed in the same experimental group of animals. The behavior of DA and NOR was in agreement with the principles of the "<u>maturation phenomenon</u>", i.e., the lowest depressions of these catecholamines were observed at 20 hour post-ischemic period following 15 minute occlusion and at 5 hours after release following 1 hour ischemia.</p>		

Project Description:

Objectives: The molecular basis of the vulnerability of brain in ischemia is not completely understood. When brain becomes severely ischemic as a consequence of thrombi, emboli or the vasospasm that can follow hemorrhage or trauma, it too probably "leaks" various intercellular constituents that normally are stored in high concentrations. The resulting inappropriate loss of several of these compounds; the monoamine neurotransmitters, norepinephrine, dopamine, and serotonin may exacerbate the pathophysiological changes caused by initial ischemia. In this report, we are presenting the behavior of biogenic amines in and following ischemia of various periods of duration.

Methods Employed: Mongolian gerbils were subjected to left common carotid occlusion and those with neurological signs of cerebral infarction were sacrificed: (a) 15 minutes, 1, 3 and 6 hours after onset of ischemia, or (b) 1 and 20 hours and 1 week after ischemia lasting 15 minutes, 1, 3 and 6 hours, respectively. Brain dopamine (DA), norepinephrine (NOR) and serotonin (5-HT) in ischemic and control brain hemispheres were assayed fluorometrically by the method of Cox and Perhach (1973). Histochemical studies of NOR and 5-HT in ischemia were done using the freeze-dried fluorescence method of Falk and Hillarp (1966) as described by Olsson and Ungerstedt (1970).

Major Findings: In 15 minute ischemic animals, no changes were found in the levels of DA, NOR and 5-HT. One hour after onset of ischemia, 5-HT level was significantly increased in ischemic hemispheres, and was not further changed up to 6 hours duration of ischemia. The levels of DA and NOR were reduced 1 hour after the occlusion was established and the reduction of these concentrations were proportional to the duration of ischemia. In recovery after ischemia, one hour after 15 minute occlusion, as well as one hour after one hour occlusion, 5-HT level was significantly higher than it was during ischemia, while in gerbils which were ischemic for longer periods, 5-HT levels were significantly lower than in ischemia. Twenty hours after the clip was removed, 5-HT level in animals which were ischemic only for 15 minutes reached the peak and was higher than one hour after the clip was removed in the same experimental group of animals. The behavior of DA and NOR was in agreement with the principles of the "maturation phenomenon", i.e. the lowest depressions of these catecholamines were observed at 20 hour post-ischemic period following 15 minute occlusion and at 5 hours after release following 1 hour ischemia. On the other hand, 5-HT level in animals clipped for 6 hours was not different from the level of the control hemisphere. The behavior of NOR in recovery in gerbils which were occluded for shorter periods (15 and 60 minutes) is different from the behavior of this catecholamine in animals which were ischemic for longer periods (3 and 6 hours). Reduced NOR level in gerbils which were ischemic for longer periods recovered after the ischemia. On the other hand, in gerbils which were ischemic for the shorter periods, NOR content showed biphasical behavior. One hour after the clip was removed NOR was higher than in controls; 20 hours after ischemia NOR level was lower than

in controls. During recovery periods, DA levels were reduced proportionally to the time of duration of ischemia. However, recovery of DA concentration was more pronounced in animals which were ischemic for the longer periods.

Significance to Biomedical Research and the Program of the Institute: These findings showed the involvement of monoamines in biochemistry of brain ischemia. In addition, data especially that of serotonin, confirmed the "maturation phenomenon." Complex alteration in biogenic amines metabolites after ischemia of short term may be expected in the period which follows the ischemic episode.

Proposed Course of the Project: This project has been completed. The resulting publication has been accepted by Acta Neuropathologica.

Publications: Mrsulja, B. B., Mrsulja, B. J., Spatz, M., Ito, U., Walker, J. T., Jr., and Klatzo, I.: Experimental cerebral ischemia in Mongolian gerbils. IV. Behaviour of biogenic amines. Acta Neuropath., 1976 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02179-02 LNNS
PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p>		
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Carbohydrates in Brain During and Following Experimental Unilateral Ischemia</p>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	B. B. Mrsulja U. Ito B. J. Mrsulja M. Spatz I. Klatzo	Visiting Scientist Visiting Fellow Visiting Fellow Head, Section on Neurocytobiol. Chief. Lab. Neuropath. Neuroanat. Sci.
LNNS NINCDS LNNS NINCDS LNNS NINCDS LNNS NINCDS LNNS NINCDS		
COOPERATING UNITS (if any) <p style="text-align: center;">None</p>		
LAB/BRANCH <p style="text-align: center;">Laboratory of Neuropathology and Neuroanatomical Sciences</p>		
SECTION <p style="text-align: center;">Section on Cerebrovascular Pathology</p>		
INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</p>		
TOTAL MANYEARS: <p style="text-align: center;">.08</p>	PROFESSIONAL: <p style="text-align: center;">.08</p>	OTHER: <p style="text-align: center;">0</p>
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> In symptoms positive groups of gerbils significant decrease of <u>glucose</u> and <u>glycogen</u> and increase of <u>lactate</u> and <u>pyruvate</u> was obtained 5 minutes after onset of ischemia in all brain structures of the hemisphere ipsilateral to the occlusion. The lowest content of glycogen was obtained 1 hour after carotic artery occlusion. Lactate and pyruvate concentrations increased and that of glucose decreased with duration of ischemia. Nine hours after onset of ischemia glycogen content was much higher than in the controls. Accumulated glycogen was histochemically observed in areas bordering the infarction. In recovery from one hour ischemia, in symptoms positive animals, levels of glucose, pyruvate and glycogen were not different from the controls 1 hour after the occlusion was removed. Lactate was significantly elevated even after 20 hours of recovery. Glycogen content was higher at that time and was elevated even 1 week after the release. </p>		

Project Description:

Objectives: The Mongolian gerbils possess a high susceptibility for the development of cerebral infarction following unilateral ligation of the common carotid artery due to frequent absence of arterial communication between the cerebral and vertebral system (Levine, S. and Payan, H., Exp. Neurol. 16: 225, 1966). The project described here was designed to evaluate the effect of ischemia on carbohydrate (glucose, glycogen, lactate and pyruvate) metabolism. In addition, the possibility of recovery of these metabolites following ischemia was approached.

Methods Employed: Mature Mongolian gerbils were subjected to the left common carotid artery clipping for 5, 15, 30 minutes, 1, 3, 5 and 9 hours. The left common carotid artery was also clipped for 1 hour and then released; the animals were sacrificed 1, 5 and 20 hours and 1 week after the release of the common carotid occlusion. All the animals with unilateral carotid artery occlusion were divided into two groups: with and without cerebral symptoms (Kahn, K., Neurol. 22: 510, 1972). The whole animals were rapidly frozen in liquid nitrogen and the following brain regions of left and right (control) hemispheres were dissected respectively: cortex, caudate, thalamus, and hippocampus. In cases of 1, 3, 5 and 9 hours of cerebral ischemia and in recovery, the brain was divided into left and right hemispheres of symptoms positive and symptoms negative animals since 1 hour of carotid occlusion induced profound changes in all investigated brain structures. Contents of glucose, lactate, pyruvate and glycogen were determined by enzymic methods spectrophotometrically. Glycogen content was also verified histochemically.

Major Findings: In symptoms positive groups of gerbils significant decrease of glucose and glycogen and increase of lactate and pyruvate was obtained 5 minutes after onset of ischemia in all brain structures of the hemisphere ipsilateral to the occlusion. The lowest content of glycogen was obtained 1 hour after carotid artery occlusion. Lactate and pyruvate concentrations increased and that of glucose decreased with duration of ischemia. Nine hours after onset of ischemia glycogen content was much higher than in the controls. Accumulated glycogen was histochemically observed in areas bordering the infarction. In groups of gerbils without neurological signs of infarction, no changes were found after 5 minutes of clipping. On the other hand, the findings similar to the symptoms positive animals, but less pronounced, were found in caudate after 15 minutes, in thalamus after 30 minutes, and in cortex and hippocampus after 60 minutes duration of occlusion. The lowest concentrations of glycogen and glucose, and the highest of lactate and pyruvate were obtained 3 hours after occlusion. The values obtained 9 hours after clipping were in the range of controls. In recovery from 1 hour ischemia, in symptoms negative gerbils, levels of glucose, lactate, pyruvate and glycogen returned to the control values during 1 hour. In symptoms positive animals, levels of glucose, pyruvate and glycogen were not different from the controls 1 hour after the occlusion was removed. Lactate was significantly elevated even after 20 hours

of recovery. Glycogen content was higher at that time and was elevated even 1 week after the release.

Significance to Biomedical Research and the Program of the Institute:
The results support the possibility of a "maturation phenomenon" which seems to be a general principle applicable to various parameters in ischemic injury. The rate of maturation of the lesions is directly related to the intensity (duration) of an ischemic insult, a lesser intensity resulting in slower development of lesions. Data for glycogen particularly supported this phenomenon. Ischemia of 1 hour duration shows accumulation of glycogen 20 hours after the clip was removed; the phenomenon observed after 9 hours of ischemia. The principle of "maturation" may be operative in clinical situations, and it could explain deterioration of the clinical conditions, in particular, after variable latent periods following ischemic insult.

Proposed Course of the Project: This project has been completed.

Publications: Mrsulja, B. B., Mrsulja, B. J., Ito, U., Walker, J. T., Jr., Spatz, M., and Klatzo, I.: Experimental cerebral ischemia in Mongolian gerbils. II. Changes in carbohydrates. Acta Neuropath. 33: 91-103, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02180-02 LNNS

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Effect of Increased Systemic Blood Pressure On Carbohydrates in Ischemic Injury

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. B. Mrsulja	Visiting Scientist	LNNS NINCDS
OTHER:	U. Ito	Visiting Fellow	LNNS NINCDS
	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

.07

PROFESSIONAL:

.07

OTHER:

0

SUMMARY OF WORK (200 words or less - underline keywords)

Glucose and glycogen were found decreased, while lactate and pyruvate increased after 1 hour of ischemic damage and except for lactate, returned to the control levels 1 hour following the ischemic episode. The recovery of metabolites was stimulated in the ischemic animals when they were under the influence of anesthesia during the recovery period. Increased systemic blood pressure did not alter the levels of carbohydrates in the control hemisphere contralateral to the occlusion. On the other hand, the recovery of glycogen, lactate and pyruvate was depressed, while that of glucose stimulated when the systemic blood pressure was increased during 1 hour recovery period and maintained at 150 mmHg.

Project Description:

Objectives: In previous studies we demonstrated that hypertensive periods account for a greatly increased incidence of blood-brain barrier damage, and it produced more severe histopathologic lesions than the ones observed in the corresponding normotensive animals. These investigations are the continuation study of influence of increased systemic blood pressure on the recovery of carbohydrates following the ischemic injury.

Methods Employed: Mature Mongolian gerbils were subjected to the common carotic artery clipping and the animals with the neurological signs of cerebral infarction (Kahn, K., Neurol. 22: 510, 1972) were: (a) sacrificed immediately after 1 hour clipping; (b) clipped for 1 hour and then the occlusion was released for 1 hour; (c) clipped for 1 hour, reanesthetized with sodium pentobarbital and sacrificed 1 hour after clip was removed; and (d) treated as the previous group, but during the release period systemic blood pressure was maintained at 150 mmHg. All animals were killed by stirring the whole animal into liquid nitrogen and left (ischemic) and right (control) hemispheres were dissected and analyzed for glucose, glycogen, lactate and pyruvate using the enzymic spectrophotometric method.

Major Findings: Glucose and glycogen were found decreased, while lactate and pyruvate increased after 1 hour of ischemic damage and except for lactate, returned to the control levels 1 hour following the ischemic episode. The recovery of metabolites was stimulated in the ischemic animals when they were under the influence of anesthesia during the recovery period. Increased systemic blood pressure did not alter the levels of carbohydrates in the control hemisphere contralateral to the occlusion. On the other hand, the recovery of glycogen, lactate and pyruvate was depressed, while that of glucose stimulated when the systemic blood pressure was increased during 1 hour recovery period and maintained at 150 mmHg.

Significance to Biomedical Research and the Program of the Institute: The results reported here show that obviously the post-ischemic elevation of the systemic blood pressure during the period of reestablished circulation has a deleterious effect on the ischemically affected brain tissue. Even though the findings presented here can't be completely equated with the cerebral ischemia in man, they might be of great clinical significance in considering the appropriate therapy.

Proposed Course of the Project: This project has been completed.

Publications: Klatzo, I., Ito, U., Go, K. G., Westergaard, E., Spatz, M. and Walker, J. T., Jr.: Experimental Brain Ischemia in Gerbils. In VIIth International Congress of Neuropathology, Budapest, Hungary. Amsterdam, Excerpta Medica, 1975, pp. 565-568.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02192-01 LNNS
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Dynamic Profiles of the Ischemic Brain Edema		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: T. Fujimoto OTHER: I. Klatzo R. M. Graham J. T. Walker, Jr.	Visiting Fellow Chief, Lab. Neuropath. Neuroanat. Sci. Biologist Biol. Lab. Tech.	LNNS NINCDS LNNS NINCDS LNNS NINCDS LNNS NINCDS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 1.0	PROFESSIONAL: .50	OTHER: .50
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>Using the <u>specific gravity</u> determinations on <u>abnormal uptake of water by the brain tissue</u> was followed in gerbils which were subjected to <u>cerebral ischemia</u> by occlusion of the common carotid artery at the neck. The significant edematous change was demonstrable in various brain structures subjected to ischemia within 10 minutes following occlusion. In the post-ischemic periods, animals which developed necrotic changes revealed extremely low specific gravity of the affected tissue and a conspicuous <u>damage of the blood-brain barrier</u>, as demonstrated by the extravasation of the Evan's blue tracer.</p>		

Project Description:

Objectives: Edema constitutes the most important complication of cerebral ischemia. Therefore, an evaluation of the mechanisms involved in ischemic brain edema is of great clinical relevance. Objective of this study is to apply a sensitive technique for demonstration of an abnormal water uptake by the brain tissue and to correlate the obtained data with the concomitant behaviour of the blood-brain barrier and histological tissue changes. This should elucidate the nature of ischemic brain edema.

Methods Employed: Mongolian gerbils are subjected to unilateral occlusion of the common carotid artery at the neck for various periods of time and sacrificed either with the remaining clip or at various time intervals following release of the occlusion. For evaluation of the blood-brain barrier (BBB) changes, the animals are injected with 2% solution of Evan's blue. Following the sacrifice by decapitation, the brains are rapidly removed and submerged in kerosene. Small samples representing the cerebral cortex, hippocampus and basal ganglia are removed from the ischemic and the opposite control hemispheres. Visible blue discoloration of the brain due to abnormal penetration of the Evan's blue into the brain tissue is recorded and photographed. The small samples are dropped into the gradient column of kerosene and bromobenzene prepared according to the method of Nelson (Nelson, S. R., Manz, M.-L., and Maxwell, J. A.: Use of specific gravity in the management of cerebral edema. *J. Appl. Physiol.* 30: 268-271, 1971). The specific gravity of the samples is recorded using standards. The corresponding brain tissue is fixed by immersion in buffered paraformaldehyde and the histological slides are prepared and stained with Cresyl Violet and H & E methods.

Major Findings: An abnormal water uptake expressed by lowering of specific gravity of the brain tissue samples was demonstrable already after 10 minutes of unreleased ischemic occlusion. The lowering of specific gravity progressed with the duration of the occlusion up to 6 hours after which it remained unchanged when estimated at 9 hr. occlusion. When the occlusion was released after 1 hr. ischemia, the amount of edema during the first 5 post-ischemic hours remained unchanged. During the 5 to 20 hour post-ischemic periods, two groups of animals could be recognized: (1) in one group the abnormal water uptake remained unchanged, and (2) the second group of gerbils showed extremely low specific gravity of the tissue on the side of occlusion. In these animals it was possible to correlate the low specific gravity values with the damage of the BBB and the histological picture of severe tissue necrosis.

Significance to Biomedical Research and the Program of the Institute: The study on ischemic brain edema is of great clinical relevance. It is obvious that an ischemic brain injury is characterized by marked heterogeneity concerning the intensity of changes, ranging from irreversibly damaged tissue to that showing only relatively slight changes. It can be assumed that by controlling the complicating edema large territories of

brain tissue affected by ischemia can be recovered and the final extent of ischemic tissue distinction can be greatly reduced. Application of some rational and effective measures for control and treatment of an ischemic insult can be possible by precise elucidation of the mechanisms involved in the development of the ischemic brain edema.

Proposed Course of the Project: The experiments will be conducted to acquire statistically meaningful data which allow establishing the dynamic profiles of ischemic brain edema and which elucidate the pathomechanisms involved.

Publications: None

1991-1992
1991-1992

1991-1992

1991-1992

1991-1992

1991-1992

1991-1992

1991-1992

1991-1992

1991-1992

1991-1992

1991-1992

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01449-10 LNNS
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PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The effect of hormones on the retrograde neuronal reaction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. Cammermeyer Head, Section on Experimental LNNS NINCDS Neuropathology

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Experimental Neuropathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland

TOTAL MANYEARS:

0.6

PROFESSIONAL:

0.1

OTHER:

0.5

SUMMARY OF WORK (200 words or less - underline keywords)

Rabbits subjected to facial nerve transection during cortisone acetate treatment display acute depression of regenerative changes in neuronal perikarya and of reactive mitosis in microglial cells. To determine whether these reactions can result in a more severe impairment of neuronal regeneration, chronic experiments have been started for quantitative studies. A comparison with thyronine-treated animals is carried out in order to establish the nature of the action of the two hormones on the central nervous system. This may have some relevance to the prognostication of clinical observations and treatment.

Project Description: .

Objectives: To determine what effect hormonal treatment may have on the recovery phases of motor neurons subjected to transection of their axons.

Methods Employed: Rabbits were treated with cortisone or thyronine for a week prior to severance of the facial nerve.

After short and prolonged survival, the animals were sacrificed and fixed by perfusion in deep anesthesia.

Serial sections of the brain stem were stained for glycogen and with PAS-galloycyanin.

Major Findings: Preliminary studies revealed that, after cortisone treatment, there is in the operated animals a depression of reactive mitosis in microglial cells and an acute depletion of glycogen in neurons. The acute neuronal reaction is the same after thyronine treatment as in untreated animals.

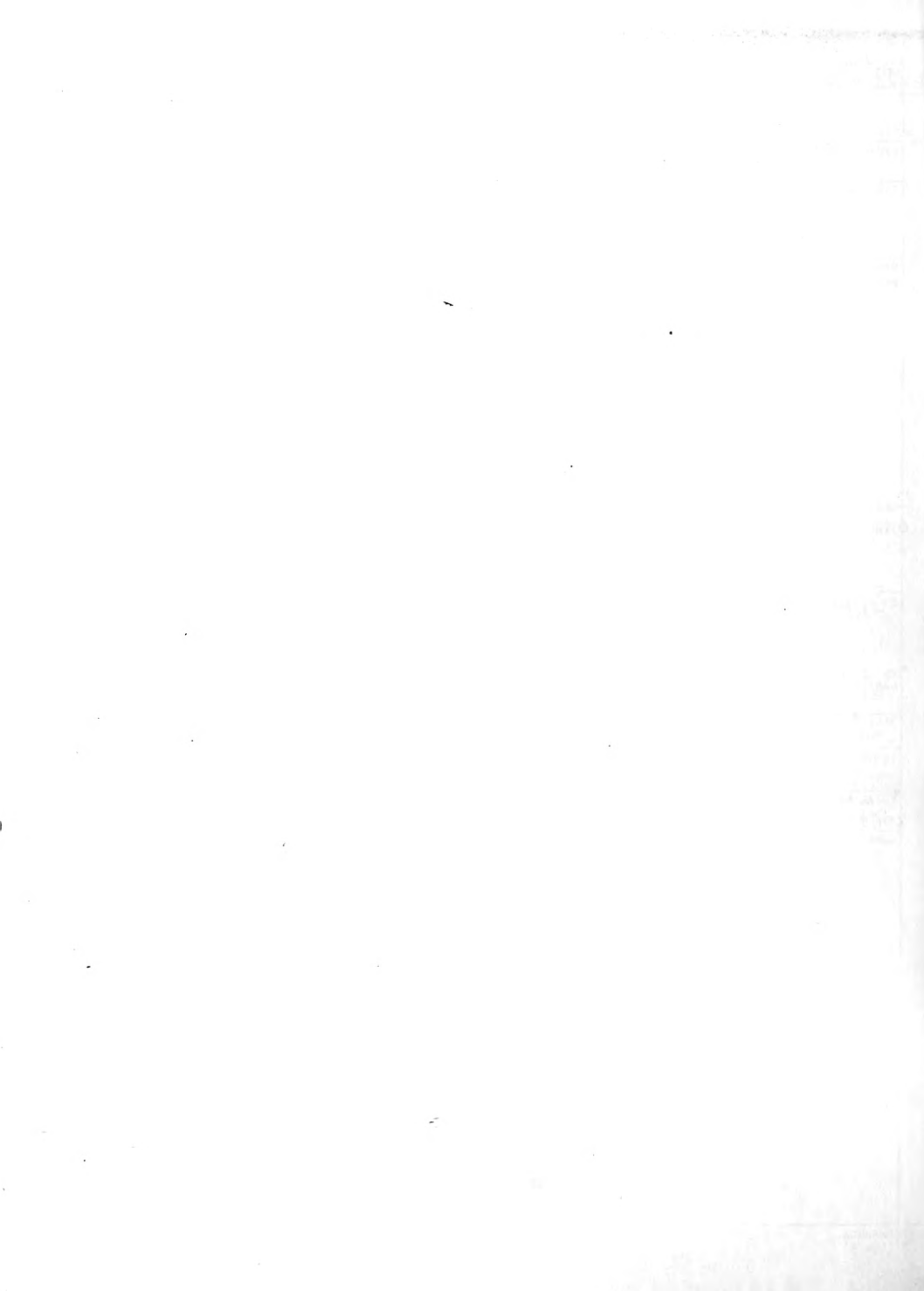
After experimental operations designed to study the chronic effect of these two hormones, no differences in clinical manifestations have been detected.

Significance: After a study on the acute depressing effect of cortisone on neuronal perikarya and neuroglial elements, it is of interest to determine whether an opposite reaction can be obtained by introduction of another hormone, such as thyronine, which is said to accelerate peripheral nerve regeneration. By counting the number of cells remaining in the facial nucleus after single operation or reoperation, one should be in a position to conclude decisively whether administration of either of the two hormones can be of any benefit or can affect the vulnerability of nervous tissue. Definite guidelines for clinical application must await results of such experiments.

Proposed Course of Project: Preparation and examination of histologic material from hormone-treated animals with prolonged survival after single transection or reoperation.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01676-08 LNNS
PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p>		
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">A comparative study of the area postrema</p>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <p style="text-align: center;">PI: J. Cammermeyer Head, Section on Experimental LNNS NINCDS Neuropathology</p>		
COOPERATING UNITS (if any) <p style="text-align: center;">None</p>		
LAB/BRANCH	Laboratory of Neuropathology and Neuroanatomical Sciences	
SECTION	Section on Experimental Neuropathology	
INSTITUTE AND LOCATION	NINCDS, NIH, Bethesda, Maryland 20014	
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0
SUMMARY OF WORK (200 words or less - underline keywords) <p>Terminated.</p>		



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02002-04 LNNS	
PERIOD COVERED July 1, 1975 to June 30, 1976					
TITLE OF PROJECT (80 characters or less) Mast cells in the brain					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT					
PI: J. Cammermeyer Head, Section on Experimental LNNS NINCDS Neuropathology OTHER: J. L. Ribas Research Scientist AFRI					
COOPERATING UNITS (if any) Department of Neurobiology, Armed Forces Radio- biology Institute, Bethesda, Maryland					
LAB/BRANCH		Laboratory of Neuropathology and Neuroanatomical Sciences			
SECTION		Section on Experimental Neuropathology			
INSTITUTE AND LOCATION		NINCDS, NIH, Bethesda, Maryland			
TOTAL MANYEARS: 0.6		PROFESSIONAL: 0.1		OTHER: 0.5	
SUMMARY OF WORK (200 words or less - underline keywords)					
<p>The functional role of <u>mast cells</u>, which are diffusely dispersed in the brain and aggregate in larger numbers in paraventricular regions, is still disputed. These cells are known to contain several biochemically active substances enabling them to prevent coagulation, control blood circulation, and take an active part in transmission of nerve impulses and in neurovegetative functions. On the basis of morphologic characteristics, the mast cells are considered to be in a labile stage of activity which can progress to exhaustion and cell death. By a quantitative study of such cell forms, indirect evidence of interaction between these cells and nervous tissue elements may be established. To this end, electron microscopic and quantitative studies have been initiated.</p>					

Project Description: ,

Objectives: To determine the significance of cytoplasmic pallor and nuclear pyknosis of mast cells in the central nervous system in an effort to clarify the functional role of these cells.

Methods Employed: Examination of microscopic sections from the brains of various animals with the aid of a specific staining technique for mast cells.

Major Findings: The appearance of mast cells varies greatly; pallor, as an expression of loss of biologically active material, is often associated with loss of the nucleus.

Significance: Since mast cells contain a variety of substances - heparin, histamine, serotonin, dopamine, etc. - which are known to act on coagulability and viscosity of blood, diameter and permeability of blood vessels and neuronal function, and since these cells occur in many parts of the brain but in varying numbers, it is assumed that their presence depends on functional requirements. If this is the case, they may play a role in controlling blood flow and neuronal interaction in the brain. The microscopic demonstration of pallor and nuclear loss may suggest that mast cells disintegrate in situ as their functional capacity is exhausted.

Proposed Course of Project: The specificity of degranulation and nuclear pyknosis in mast cells is to be investigated in serial sections of brain stem in different animal species.

In another investigation, started in collaboration with Dr. J. L. Ribas, the electron microscopic characteristics of mast cells in different functional stages will be ascertained.

Publications:

Cammermeyer, J.: Factors contributing to denucleation of cerebral mast cells. Acta Anat. (in press).

Project Description:

Objectives: To subject the taenia thalami in cat to an ultramicroscopic survey because of its prominence in this species.

Methods Employed: The variety of elements in this region have been identified by conventional light microscopic techniques.

Major Findings: A region rich in blood vessels and collagenous fibers, nerve cells, supporting glial elements, plasma cells and mast cells occupies the taenia thalami of the cat.

Significance: On the basis of its complex structure, it has been speculated that this region may serve a chemoreceptive or baroreceptive function.

Proposed Course of Project: To examine the submicroscopic composition of the thalamo-choroidal body.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02004-04 LNNS
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) The pathogenesis of myelopathies		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: J. Cammermeyer Head, Section on Experimental LNNS NINCDS Neuropathology		
COOPERATING UNITS (if any) None		
LAB/BRANCH	Laboratory of Neuropathology and Neuroanatomical Sciences	
SECTION	Section on Experimental Neuropathology	
INSTITUTE AND LOCATION	NINCDS, NIH, Bethesda, Maryland 20014	
TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0
SUMMARY OF WORK (200 words or less - underline keywords) The volumetric manifestation of edema or swelling of the <u>spinal</u> <u>cord</u> will be explored in animal <u>material</u> in order to clarify factors involved in the pathogenesis of acute myelopathies and to formulate methods for their prevention and cure.		

Project Description:

Objectives: To elucidate the mechanism of spinal cord lesions.

Methods Employed: For an orientation of the characteristics of edema within the spinal cord, the volume of each segment of the spinal cord is measured prior to and after immersion in water for varying lengths of time.

Major Findings: (1) When the spinal cord is immersed in fluids, the degree of swelling varies with tonicity of the solute as well as with region.

(2) Splitting of the surrounding membranes demonstrated a far greater swelling which in the intact spinal cord is restricted by the membranes.

Significance: In some preliminary reviews, it was found that the human spinal cord becomes firmer during swelling because of the restrictive action of the superficial membranes, a reaction also noticeable in the cat spinal cord.

Since resistance to swelling by the unyielding membranes results in abnormal blood circulation, edema can cause spinal cord lesions. Knowledge of the role of edema in the pathogenesis of spinal cord diseases is important for the establishment of guidelines for treatment and prevention of spinal cord edema occurring as a complication to a number of human diseases, such as trauma, viral infection (poliomyelitis), allergic manifestations (multiple sclerosis), cancer, etc. The results of post-mortem experiments purporting to mimic intravital processes will serve as a foundation for additional experimental investigations.

Proposed Course of Project: To define criteria whereby morphologic evidence of compromised blood flow can be determined.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02143-02 LNNS
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Volumetric changes of brains during histologic preparation		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: J. Cammermeyer Head, Section on Experimental LNNS NINCDS Neuropathology		
COOPERATING UNITS (if any) None		
LAB/BRANCH	Laboratory of Neuropathology and Neuroanatomical Sciences	
SECTION	Section on Experimental Neuropathology	
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0
SUMMARY OF WORK (200 words or less - underline keywords) For the purpose of controlling changes in <u>volume</u> of the <u>brain</u> during fixation, a special device has been designed and a baseline of volumes for different species has been established.		

Project Description:

Objectives: To measure the volume of brains from small animals in order to test the effect of fixation and dehydration.

Methods Employed: An apparatus was designed for direct measurement of small brains utilizing the same principle as laid down earlier in the design of an apparatus for volumetric measurement of segments of the intact spinal cord. Simultaneous measurement of the brain weight permitted calculation of the specific gravity.

Major Findings: When brains of animals of different species were fixed by perfusion first with saline and then with Bouin's solution, their specific gravity remained constant.

The volume of the Bouin-fixed brains was unaffected by flow, rate, amount or temperature of the fixative.

Significance: For standardization of the histologic preparation of the brain, it is important that volume of tissues be controlled. Constancy in volume reflects adequacy of the preparatory technique, a requirement for estimating the status of the neurons. The results obtained may provide a baseline for assessing the effect of experimentally produced damage or edema in the brain.

Proposed Course of Project: To compare the changes in volume of brains during the process of alcohol dehydration after fixation by immersion and by perfusion.

Publications: None

Project Description:

Objectives: To assess the morphologic characteristics, source and mode of development of fat emboli in normal animals fixed by perfusion.

Methods Employed: Animals were fixed by perfusion, and oil red O fat-stained frozen sections were prepared from representative blocks of the brains.

Major Findings: In approximately 25% of the normal rabbits and cats fixed by perfusion, a few isolated fat emboli occur in veins and arteries near the cerebral surface. Their formation was found to depend on accumulation of tissue fat on the brain surface during the autopsy, tearing of the leptomeninges and superficial cerebral blood vessels, and suction caused by stretching and constriction of blood vessels during removal of the brain. When the brain is exposed under continuous flow of water, the development of such false emboli can be prevented.

For comparison, oil was injected into blood vessels and the animals were sacrificed shortly thereafter. Such fat emboli in perfused-fixed material abut columns of closely packed erythrocytes. After prolonged intervals between injection and perfusion, tissue changes of different types develop. The absence of any such changes in the normal perfused-fixed animals indicates that the isolated emboli in these animals had not circulated during life.

Significance: The observation that fat emboli occur in perfused-fixed normal material invalidates the diagnostic value of such vascular occlusions unless the autopsy was performed under special precautions by the application of running water. The occurrence of single or isolated fat emboli in empty vascular channels can not be taken as a sign of intra-vital circulation in material fixed by perfusion; even in material fixed by immersion, similar precautions during removal of the brain should be followed in order to assure unequivocal diagnosis. The simultaneous occurrence of fat and erythrocytes is an important criterion of intra-vital transport of fat.

Proposed Course of Project: To determine the occurrence of fat emboli in different laboratory animals fixed by perfusion and to develop methods to prevent formation of such emboli.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02194-01 LNNS	
PERIOD COVERED July 1, 1975 to June 30, 1976					
TITLE OF PROJECT (80 characters or less) Fat emboli in normal flattened retina fixed by perfusion					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: J. Cammermeyer Head, Section on Experimental LNNS NINCDS Neuropathology					
COOPERATING UNITS (if any) None					
LAB/BRANCH		Laboratory of Neuropathology and Neuroanatomical Sciences			
SECTION		Section on Experimental Neuropathology			
INSTITUTE AND LOCATION		NINCDS, NIH, Bethesda, Maryland			
TOTAL MANYEARS: 0.6		PROFESSIONAL: 0.2		OTHER: 0.4	
SUMMARY OF WORK (200 words or less - underline keywords) The unexpected demonstration of fat emboli in flattened retina of perfused-fixed animals is associated with influx of extraneous fat during removal of the eye and preparation of the flattened retina. Embolization of this type can be prevented by <u>ligating the optic nerve</u> prior to removal of the eye.					

Project Description:

Objectives: To determine the frequency, origin and diagnostic criteria of fat emboli in the retina of animals fixed by perfusion.

Methods Employed: In animals fixed by perfusion, the optic nerve was ligated and the retina carefully separated from the sclera. The loosened retina, after staining with oil red O, was flattened on a slide and covered with glycerin gelatin.

Major Findings: On rare occasions, fat emboli were demonstrable in the flattened retina of cats which had been fixed by perfusion. The emboli occluded larger blood vessels but did not usually enter into the smallest blood vessels or capillaries. The blood vessels were free of erythrocytes. After injection of oil, the fat emboli occluding numerous blood vessels formed a wide-meshed network of embolized capillaries. In order to prevent formation of the emboli, the optic nerve was ligated prior to removal of the eye and during flattening of the retina. The corpus vitreum contained large amounts of fat, which should be flushed away with running water.

Significance: Since fat emboli in normal retina fixed by perfusion can be avoided by ligation of the optic nerve, as well as by ample flushing with running water, they are regarded as artifactual in nature. The realization that such emboli may complicate experimental material is important for a correct interpretation of morphologic observations and for the unequivocal diagnosis of retinal fat embolism.

Proposed Course of Project: To develop techniques whereby formation of false fat emboli is prevented and to determine their occurrence in different animals.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02195-01 LNNS
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Perivascular cerebral siderosis as a marker of blood-brain barrier damage		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: J. Cammermeyer Head, Section on Experimental LNNS NINCDS Neuropathology		
COOPERATING UNITS (if any) None		
LAB/BRANCH	Laboratory of Neuropathology and Neuroanatomical Sciences	
SECTION	Section on Experimental Neuropathology	
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland		
TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.2	OTHER: 0.7
SUMMARY OF WORK (200 words or less - underline keywords) In the course of a study on fat embolism in the cat, a focal <u>diffusion of iron</u> was demonstrated ubiquitously through the <u>brain at sites of damaged blood-brain barrier</u> . The role of <u>changes in serum iron</u> concentration in the diffusion of iron is being explored in experiments on different animal species. Under such conditions, it should be possible to determine whether iron has any toxic effect on tissue elements.		

Project Description: .

Objectives: To determine the source of focal deposition of iron.

Methods Employed: Cerebral fat embolism is induced by intravenous, intracardial and intraperitoneal injection of oil. Serum iron levels are determined by the Technicon Auto-Analyzer method. The autopsy is performed with precautions to prevent introduction of extraneous fat. Histologic material consists of frozen sections stained with oil red O and paraffin sections stained by the Turnbull blue method for iron and other methods for study of different elements.

Major Findings: In animals injected with oil into the systemic circulation, there are, ubiquitously through the brain, multiple foci with iron deposited in astrocytes, microglial cells and oligodendrocytes. These foci are more numerous than the lesions observed in PAS-gallocyanin-stained sections in which a loss of nerve cells and nerve fibers, progressive changes of astrocytes and proliferative changes of microglial cells are demonstrable.

In a pilot survey, analysis of serum iron has disclosed changes in concentration of circulating iron following injection of oil, suggesting that serum iron may be the source of perivascularly deposited iron.

Significance: In animals, as well as in man, because of the normal content of serum iron in the blood, the diffusion of iron acts as an inherent marker of blood-brain barrier damage. The demonstration of such damage can then be determined under conditions when it is inadvisable to inject acid semicolloidal dyes, generally used in experiments to reveal any vascular damage. In addition, the toxic effect of perivascularly deposited iron on tissue elements can be estimated and factors contributing to ferruginization of neuronal and glial elements can be explored.

Proposed Course of Project: To examine histologically material from different laboratory animals subjected to serum iron analysis following injection of oil, in order to determine (a) the severity of serum iron changes in the blood (dog), (b) when they may occur after injection of oil (cat), and (c) whether the experimental changes are influenced by the age of the animal (monkey).

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01442-10 LNNS
PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p>		
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Permeability of Cellular Layers in the Vertebrate Nervous System</p>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: T. S. Reese OTHER: Robert Gulley Lise Prescott M. W. Brightman	Head, Sect. on Functional Neuroanat. Sr. Staff Fellow Guest Worker Head, Sect. on Neurocytology	LNNS NINCDS LNO NINCDS LNNS NINCDS LNNS NINCDS
COOPERATING UNITS (if any) <p style="text-align: center;">Laboratory of Neuro-Otolaryngology, NINCDS</p>		
LAB/BRANCH <p style="text-align: center;">Laboratory of Neuropathology and Neuroanatomical Sciences</p>		
SECTION <p style="text-align: center;">Section on Functional Neuroanatomy</p>		
INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</p>		
TOTAL MANYEARS: <p style="text-align: center;">0.2</p>	PROFESSIONAL: <p style="text-align: center;">0.1</p>	OTHER: <p style="text-align: center;">0.1</p>
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> The location and nature of <u>extracellular barriers</u> to colloidal substances at a variety of locations in the nervous system was determined. At the <u>meningeal</u> covering of the brain, at the special <u>ependyma</u> covering the <u>median eminence</u> and <u>area postrema</u>, and at the reticular lamina in the <u>organ of Corti</u>, extensive systems of tight junctions form continuous bands surrounding cells and occluding extracellular spaces. It is these bands of junctions which control the movement of colloidal and probably ionic substances in intervening extracellular spaces. No tight junctions are found at the <u>astrocytic border</u> of the brain but other membrane specializations revealed by the <u>freeze-fracture technique</u> suggest that these cells also participate in the <u>blood-brain barrier</u> system. These data on the cellular basis of brain barriers, as well as the new structural approaches used in these studies, afford a basis to design experimental studies of a variety of clinically important conditions which depend on or produce damage to brain barriers. They also give rise to new ideas about the roles of various cells associated with neurons and about the function of barrier systems in special regions, such as the organ of Corti. </p>		

Project Description:

Objectives: Determination of the structural basis of biologically and clinically important barriers by studying the pathways taken by colloids and large molecular weight solutes to cross the various cellular layers associated with the nervous system. Currently being studied are: (1) ependyma covering the median eminence and area postrema; (2) arachnoid-dural membrane; (3) glial borders of the brain; and (4) organ of Corti in the inner ear.

Methods Employed: Various methods are used to prepare brains of laboratory animals for examination with the electron microscope. The cytochemical method of Karnovsky is used to localize purified horseradish peroxidase (MW 40,000) or microperoxidases (MW less than 2000) after they are injected into the blood or cerebrospinal fluid. Junctions between cells bordering these compartments are examined with the electron microscope after applying special stains for intercellular junctions. The freeze-fracture technique has become of major importance to determine the structure and deployment of these junctions.

Major Findings: Peroxidase injected into the cerebrospinal fluid of the subarachnoid space in the mouse is able to pass between cells of the pia-arachnoid layer, cross the basement lamina of the brain, and pass between the astrocytic processes forming the limiting surface of the brain to reach typically open interstitial spaces of the brain. However, peroxidase does not cross from ventricular cerebrospinal fluid into the median eminence and area postrema. The basis of this selective barrier is a system of tight junctions within the specialized epithelial cells lining the ventricular surface over these regions.

Unlike most regions of the brain, the median eminence lacks a blood-brain barrier (BBB) at the walls of its blood vessels. However, it is isolated from the cerebrospinal fluid (CSF) by a specialized ependyma which acts as a barrier between its parenchyma and the overlying CSF. Similarly, the dural covering of the brain lacks a BBB but embedded in the underlying arachnoid is a specialized "barrier layer" of cells between the dura and the CSF. This barrier layer of the arachnoid is missing at arachnoid villae where CSF is reabsorbed. The conclusion which emerges from these studies is that the brain and CSF are separated from the blood by a continuous layer of cells which act as a barrier to proteins. This barrier layer is absent at only a few locations for specific purposes such as reabsorption of CSF.

Other studies of the blood-brain barrier, using the freeze-fracture technique, permitted a more detailed look at the limiting astroglial membrane of the brain where it faces the CSF. Although there is no barrier to proteins in this layer, a complex system of intercellular junctions and other specializations of the astrocytic membranes were discovered. While this significance to the BBB system is not clear yet, this finding focuses attention on the astroglia as possibly having a subtle role in the blood-brain barrier system.

Barriers in the organ of Corti of the mammalian inner ear were the object of a collaborative study with Dr. Robert Gulley. The perilymph is separated from the endolymphic fluid by tight junctions between the apices of hair and supporting cells. The freeze-fracture technique was used to examine these junctions and they are more extensive, and presumably, "tighter" than in any other tissue studied so far. Their unique form also suggests that they might have functions other than to prevent mixing of endolymph and perilymph and, perhaps, are involved in the first steps in the sensing of mechanical displacements in the organ of Corti.

Significance to Biomedical Research and the Program of the Institute: We are determining the permeability to small proteins of special cellular layers associated with blood vessels and meninges of the blood vessels and brain surfaces. Our determinations depend on cytological techniques that show specifically which components of which layers are permeable. Thus we are able to determine the cells as well as the probable mechanisms that control the chemical environment of the brain (blood-brain barrier and blood-cerebrospinal fluid barriers). By applying this knowledge it is possible to determine the changes in cell and cell membrane structure which are responsible for pathological changes in the brain barrier system, such as those which occur during acute experimental meningitis. Also of interest is that our recent data indicates a possible role for astrocytes in the brain barrier system. This possibility will focus attention on the role of these glial cells in pathological conditions affecting the brain barrier system.

These data on the cellular basis of the BBB, as well as the anatomical techniques used in these studies, afford a basis to design experimental studies of a variety of clinical disorders which depend on or produce disruption of the BBB. They also give rise to new ideas about the roles of the various cells associated with neurons and about the function of barrier systems in special regions such as the organ of Corti.

Proposed Course of the Project: The work on the arachnoid membrane has been completed and published. The work on the glial membranes, done in conjunction with Project No. Z01 NS 02145-02 LNNS, has been published. The next step is to find a culture preparation where these membranes can be studied in vitro and their components isolated. We do not have plans to attempt this in the next year. The work on the organ of Corti has been completed and is being published. We expect to spend little time on this project in the next year. The paper on the median eminence was done in conjunction with Project No. Z01 NS 02145-02 LNNS.

Publications: Brightman, M. W., Prescott, L., Reese, T. S.: Intercellular junctions of special ependyma. In Knigge, L. M., Scott, D. E., Kobayashi, H., and Ishii, S. (Eds.): Brain-Endocrine Interaction II. The Ventricular System in Neuroendocrine Mechanisms. Basel, Karger, 1975, pp. 146-165.

Brightman, M. W., and Reese, T. S.: Membrane specializations of ependymal cells and astrocytes. In Tower, D. B. (Ed.): The Nervous System, Vol. I: The Basic Neurosciences. New York, Raven Press, 1975, pp. 267-277.

Nabeshima, S., Reese, T. S., Landis, D. M. D., and Brightman, M. W.: Junctions in the meninges and marginal glia. J. Comp. Neurol. 164: 127-169, 1975.

Gulley, R. L., and Reese, T. S.: Intercellular junctions in the reticular lamina of the organ of Corti. J. Neurocytol. 1976 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER <div style="text-align: right;">Z01 NS 01881-06 LNNS</div>																																								
PERIOD COVERED <div style="text-align: center;">July 1, 1975 to June 30, 1976</div>																																										
TITLE OF PROJECT (80 characters or less) <div style="text-align: center;">Structural Basis of Synaptic Transmission</div>																																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																																										
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">T. S. Reese</td> <td style="width: 40%;">Head, Sect. on Functional Neuroanat.</td> <td style="width: 10%;">LNNS NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>Maryanne Henkart</td> <td>Sr. Staff Fellow</td> <td>BBB NICHD</td> </tr> <tr> <td></td> <td>Rosemary Rees</td> <td>Visit. Fellow</td> <td>LNNS NINCDS</td> </tr> <tr> <td></td> <td>David Pumplin</td> <td>Biologist</td> <td>LNNS NINCDS</td> </tr> <tr> <td></td> <td>Mary Rheuben</td> <td>Biologist</td> <td>LNNS NINCDS</td> </tr> <tr> <td></td> <td>Robert Gulley</td> <td>Sr. Staff Fellow</td> <td>LNO NINCDS</td> </tr> <tr> <td></td> <td>John Heuser</td> <td>Dept. Physiol.</td> <td>UCSF</td> </tr> <tr> <td></td> <td>Dennis Landis</td> <td>Dept. Neurol.</td> <td>Mass. Gen. Hosp.</td> </tr> <tr> <td></td> <td>Avery Nelson</td> <td>Guest Worker</td> <td>LNNS NINCDS</td> </tr> <tr> <td></td> <td>M. Dubois-Dalcq</td> <td>Chief, Sect. Electron Micros.</td> <td>IDB NINCDS</td> </tr> </table>			PI:	T. S. Reese	Head, Sect. on Functional Neuroanat.	LNNS NINCDS	OTHER:	Maryanne Henkart	Sr. Staff Fellow	BBB NICHD		Rosemary Rees	Visit. Fellow	LNNS NINCDS		David Pumplin	Biologist	LNNS NINCDS		Mary Rheuben	Biologist	LNNS NINCDS		Robert Gulley	Sr. Staff Fellow	LNO NINCDS		John Heuser	Dept. Physiol.	UCSF		Dennis Landis	Dept. Neurol.	Mass. Gen. Hosp.		Avery Nelson	Guest Worker	LNNS NINCDS		M. Dubois-Dalcq	Chief, Sect. Electron Micros.	IDB NINCDS
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INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014																																										
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																																								
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SUMMARY OF WORK (200 words or less - underline keywords)																																										
<p> This project seeks to determine the location and mechanism of <u>transmitter secretion</u> and reception at <u>synapses</u>. In order to capture the fleeting structural changes which accompany <u>discharge of synaptic vesicles</u>, methods for <u>rapid freezing</u> synapses have been developed. Structural changes are then visualized at the cell membrane level by preparing the frozen tissues with <u>freeze-fracture technique</u>. The freeze-fracture technique also reveals structural details which may be specific for different pharmacological types of synapses; this relationship is being investigated by examining the structure of the <u>post-synaptic membrane</u> in different types of synapses. New methods are also being tried for marking specific chemical activities, such as <u>receptors</u>, to identify them in freeze-fracture, as well as conventional thin section preparations. The assembly of <u>viral membranes</u> is being used as a simple model with which to develop these techniques. The significance of this work is that it defines the normal structure of synapses and relates normal variations in structure to different functional states. Thus, it becomes possible to distinguish pathological changes in structure, which is an issue of great importance in looking for the etiology of epilepsy or myasthenia gravis with structural techniques. </p>																																										

Project Description:

Objectives: Synapses are sites where electrical signals pass between neurons or between neurons and muscle cells. This project seeks to find the exact location and mechanism of synaptic transmission in the central and peripheral nervous systems.

Methods Employed: Synapses are prepared for examination in the electron microscope by the freeze-fracture technique in order to visualize the internal structure of their surface membranes. The vertebrate nerve-muscle synapse has been the main object of study in the last year, but nerve muscle synapses from insects have also been examined because they use glutamate rather than acetylcholine as a neurotransmitter. Isolated muscles are exposed to a variety of different ionic environments in conjunction with different schedules of electrical nerve stimulation, black widow spider venom, and botulinum toxin. They are then put in an aldehyde fixative, or frozen in a special apparatus designed in our laboratory, in order to capture fleeting structural changes associated with synaptic vesicle discharge and recovery. They are then freeze-fractured and the resulting replicas of split membranes examined in a high resolution electron microscope.

Major Findings: This year we published a method for capturing the fleeting structural details in functioning synapses by freezing them very rapidly, thereby avoiding the use of chemical fixatives and cryoprotective agents which alter profoundly the structure of synapses. By combining this method with the freeze-fracture technique we have been able to see how synaptic vesicles fuse with the surface of the synapse to discharge their contents. Also, we have recognized particulate components of the synaptic vesicle wall which are incorporated into the surface membrane during synaptic activity to be later recovered and incorporated into new synaptic vesicles. This finding of particle recycling confirms earlier work which showed that local recycling of synaptic vesicles replaces those lost during synaptic activity.

Freeze-fracturing is now being applied to see whether exocytosis and membrane recycling occur in different pharmacological types of synapses. So far, the results at synapses using glutamate (or aspartate) as a transmitter are consistent with the conclusions above. We are also exploring the effects of botulin toxin and black widow spider venom on synapses. So far, the results indicate that the toxin specifically blocks exocytosis, probably by preventing calcium ions from entering the nerve terminal, and that the venom lets calcium ions in to excite exocytosis. One of the most interesting findings, which supports these conclusions, is that the venom can overcome the effect of the toxin, so that the normal distribution of exocytosis reappears. The clinical usefulness of this finding is limited, however, unless a way could be found to return the exocytosis to control by nerve impulses arriving at the synapse.

The freeze-fracture technique has yielded new information about the structure of the post-synaptic membrane. Particulate structures, thought to be receptor molecules within the post-synaptic membrane, appear to be specific for different types of synapse. This year, new information has indicated that these particle aggregates are specific for each chemical and functional type of synapse. Nicotinic receptors at excitatory cholinergic synapses always have the same structure, regardless of the species of animal examined, but presumed cholinergic synapses in the middle ear, which are thought to be inhibitory, have a different type of post-synaptic specialization. Conversely, neuromuscular synapses, which are excitatory, have different types of post-synaptic specializations depending on whether acetylcholine or glutamate is the transmitter. While more chemical types of synapses need to be examined, these studies indicate that identification of pharmacological types of synapses might be approached with anatomical techniques.

A major effort has recently been made to develop new techniques for examining the cell surface at a molecular level. These efforts have been successful and have been applied to a study viral budding in tissue culture in collaboration with Dr. Monique Dubois-Dalcq, Infectious Diseases Branch, NINCDS (Project No. Z01 NS 02034-04 ID). We hoped to see how neurotrophic viruses form in infected cells but also expected that infected cells would serve as good experimental subjects with which to develop methods to see how components of the membranes are assembled in special regions of their surface. For these purposes, we developed techniques for seeing surface details as small as single protein molecules, and a way to tag these surface sites with antibodies. These techniques made it possible to see how viral membrane is assembled and with this knowledge it was possible to pinpoint the steps which are missing in chronic (slow) viral infections with such agents as the strain of measles virus which causes subacute sclerosing panencephalitis. The same techniques are now being applied to cultured neurons to see how the post-synaptic membrane at synapses is assembled, a problem which is central to understanding both the development and regeneration of synapses.

Significance to Biomedical Research and the Program of the Institute:
One of the most immediately practical aspects of the studies on synapses is that they define the normal structure of various types of synapses in a variety of functional states. This knowledge will permit distinction between normal and pathological, as well as between resting and active synapses, with the electron microscope. In structural studies of epileptic brains, it should now be possible to distinguish normally active from pathologically active synapses. Similarly, in diseases involving peripheral nerve-muscle synapses at neuromuscular junctions, it will be possible to distinguish pathological states from changes resulting from increased or decreased activity. The finding that different chemical types of synapses are distinguished by the freeze-fracture technique may contribute to the task of determining the chemical organization of synapses in the central nervous system. Knowledge about the locations and pharmacological types of various central nervous system synapses will make it possible to understand the action of drugs on the brain on a cellular level.

Proposed Course of the Project: The work on the organ of Corti has been completed and submitted for publication. The method for rapid freezing is now reliable and has been published. The work on insect neuromuscular junctions and the work with venoms and toxins will be presented at the Neuroscience and Cell Biology Meetings. The work on viral budding, in both productive and chronic infections, is in the process of publication. In the next year, we plan to use the rapid freezing method to look at membrane fusion, using discharge of synaptic vesicles and fusion of virus with the host cell membrane as test systems. We hope the work on neurotoxins, on insect neuromuscular junctions, and on frog sympathetic ganglion synapses can be finished and prepared for publication next year.

Publications: Dubois-Dalcq, M., and Reese, T. S.: Structural changes in the membrane of vero cells infected with a paramyxovirus. J. Cell Biol. 67: 551-565, 1975.

Heuser, J. E., Landis, D. M. D., and Reese, T. S.: Preservation of rapidly changing structures in synapses by rapid freezing. Cold Spring Harbor Symposium on the Synapse, 1975 (in press).

Dubois-Dalcq, M., Reese, T. S., Murphy, M. and Fuccillo, D.: Defective bud formation in human cells chronically infected with SSPE virus. J. Virol., 1976 (in press).

Henkart, M., Landis, D. M. D., and Reese, T. S.: Similarity of junctions between plasma membranes and endoplasmic reticulum in muscle and neurons. J. Cell Biol., 1976 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01880-06 LNNS																
PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p>																		
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Hyperosmolar and $HgCl_2$ Effect on the Brain Uptake of ^{14}C Glucose Analogues</p>																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%; vertical-align: top;"> PI: </td> <td style="width: 30%; vertical-align: top;"> M. Spatz </td> <td style="width: 30%; vertical-align: top;"> Head, Section on Neurocytobiol. </td> <td style="width: 25%; vertical-align: top;"> LNNS NINCDS </td> </tr> <tr> <td style="vertical-align: top;"> OTHER: </td> <td style="vertical-align: top;"> I. Klatzo </td> <td style="vertical-align: top;"> Chief, Lab. Neuropath. Neuroanat. Sci. </td> <td style="vertical-align: top;"> LNNS NINCDS </td> </tr> <tr> <td></td> <td style="vertical-align: top;"> Z. M. Rap </td> <td style="vertical-align: top;"> Guest Worker </td> <td style="vertical-align: top;"> Polish Acad. Sci. </td> </tr> <tr> <td></td> <td style="vertical-align: top;"> S. I. Rapoport </td> <td style="vertical-align: top;"> Neurophysiologist </td> <td style="vertical-align: top;"> LNP NIMH </td> </tr> </table>			PI:	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS	OTHER:	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS		Z. M. Rap	Guest Worker	Polish Acad. Sci.		S. I. Rapoport	Neurophysiologist	LNP NIMH
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COOPERATING UNITS (if any) <p style="text-align: center;">Polish Academy of Sciences, Warsaw, Poland Laboratory of Neurophysiology, NIMH</p>																		
LAB/BRANCH <p style="text-align: center;"><u>Laboratory of Neuropathology and Neuroanatomical Sciences</u></p>																		
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INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</p>																		
TOTAL MANYEARS: <p style="text-align: center;">0</p>	PROFESSIONAL: <p style="text-align: center;">0</p>	OTHER: <p style="text-align: center;">0</p>																
SUMMARY OF WORK (200 words or less - underline keywords) <p style="text-align: center;">This project has been discontinued for the present time.</p>																		

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01999-04 LNNS
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Uptake of Sucrose, Dextrose and Albumin in Ischemia		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: T. Fujimoto OTHER: M. Spatz I. Klatzo	Visiting Fellow Head, Section on Neurocytobiol. Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS LNNS NINCDS LNNS NINCDS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 1.25	PROFESSIONAL: .75	OTHER: .50
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> The passage of small and large radiolabeled molecules with and without Evan's blue marker from blood to brain has been investigated in order to elucidate the <u>blood-brain barrier</u> permeability and its relationship to the development of <u>ischemic cerebral edema</u>. This investigation is still in progress. The preliminary results are as follows: (A) Long-term ischemic gerbils subjected to cerebral ischemia of 3 hours and 15 minutes recovery period showed only an increase in sucrose and dextrose values in the affected hemisphere whenever extravasation of Evan's blue albumin complex was seen in the cerebral tissue. Three hours after reestablishment of cerebral circulation following ischemia of 3 hours duration, the cerebral levels of dextrose were found increased only in animals with increased BBB permeability to Evan's blue. However, the cerebral sucrose levels were increased irrespective of presence or absence of increased permeability blood-brain barrier to Evan's blue. (B) So far, <u>RISA</u> was investigated only in short-term ischemia of 1 hour and various recovery periods showing an increased level after 20 hours of recovery in two out of six animals. </p>		

Project Description:

Objectives: Cerebral edema was found to be the most common feature in gerbils subjected to various periods of cerebral ischemia (Ito, U., Spatz, M., Walker, J. T., Jr., and Klatzo, I., *Acta Neuropath.* 32: 209-223, 1975). Moreover, in short-term ischemia, a biphasic change in cerebral water content was observed in the affected hemisphere which appears to be related to the absence or presence of increased blood-brain barrier (BBB) permeability after the cerebral circulation was reestablished (unpublished observation). Therefore, this study was undertaken to investigate the passage of small and large radiolabeled molecules with and without Evan's blue from blood to brain in order to elucidate the functional status of the BBB and possible mechanism responsible for the development of cerebral ischemic edema.

Methods Employed: Several groups of gerbils were subjected to 1 and 3 hours common carotid clipping and various periods of clip release. Only animals with neurological signs were injected with the radiolabeled substances with and without Evan's blue via femoral vein 20 minutes prior to the termination of the experimental period. After decapitation, the brains were removed quickly and each hemisphere was divided into two parts. The cerebral samples were weighed and processed for liquid scintillation counting or gamma counting.

Major Findings: This investigation is in progress. So far, the following observations were made: (1) no differences were observed in the cerebral level of RISA between the affected and unaffected, as well as from control hemisphere, 1 and 5 hours after clip release of 1 hour duration. Twenty hours after the cerebral circulation was reestablished following 1 hour occlusion, two out of six animals showed a 30% higher level of RISA in the hemisphere ipsilateral as compared to the hemisphere contralateral to arterial clipping. The animals subjected to cerebral ischemia for 3 hours and 15 min. recovery period showed an increased sucrose and dextrose value in the affected hemisphere only whenever the Evan's blue extravasation was present in the cerebral tissue. Three hours after the cerebral circulation was reestablished following 3 hours of ischemia, the cerebral level of dextrose were found increased in animals with increased BBB permeability to Evan's blue albumin complex. However, all animals showed a 40% increased sucrose level in the ischemic hemisphere irrespective of the presence or absence of increased BBB permeability to Evan's blue.

Significance to Biomedical Research and the Program of the Institute: The basic comprehension of the blood-brain barrier functions concerned with the passage of nutrient and non-nutrient substances from blood to brain following cerebral ischemia is of major importance (1) for the understanding of the mechanism responsible for the development of ischemic edema, as well as elucidating other pathophysiological processes occurring in cerebrovascular disease and many other neurological disorders, and (2) for selecting the best therapeutic approach to a given disease.

Proposed Course of the Project: This model will be useful to study the facilitated transport of different substances, which may be altered in ischemic edema.

Publications: None

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PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Brain Edema in Cerebral Ischemia of Gerbils		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	T. Fujimoto	Visiting Fellow LNNS NINCDS
OTHER:	M. Spatz	Head, Section on Neurocytobiol. LNNS NINCDS
	H. Pappius	Assoc. Prof. Neurochem. Montreal Neuro. Inst.
	K. G. Go	Assoc. Prof. Neurosurgery Univ. Groningen
	I. Klatzo	Chief, Lab. Neuropath. LNNS NINCDS
		Neuroanat. Sci.
COOPERATING UNITS (if any) Montreal Neurological Institute, Montreal, Quebec, Canada Dept. of Neurosurgery, University of Groningen, Groningen, The Netherlands		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: .87	PROFESSIONAL: .75	OTHER: .12
SUMMARY OF WORK (200 words or less - underline keywords) Experimentally, cerebral <u>ischemic edema</u> can be produced easily in 30% of Mongolian gerbils by unilateral ligation of the common <u>carotid artery</u> . The cerebral water content was assessed by the determination of <u>wet</u> and <u>dry weight</u> and the <u>specific gravity</u> of the tissues. In short-term ischemia of 1 hour duration, the cerebral water content was increased, but did not show any progression until 10 hours after the release of arterial occlusion. At this time, the percent of water content was more pronounced coinciding with the increased permeability of blood-brain barrier to Evan's blue tracer. A week later only half of the animals showed recovery. In long-term ischemia, progressive accumulation of water content was observed with prolonged duration of ischemia.		

Project Description:

Objectives: In human cerebral ischemia, brain edema is considered to be an important factor in causing mortality (Shaw, C., Alvord, E., and Berry, R., Arch. Neurol. 1: 161-177-1959). Experimentally, cerebral ischemia can be easily produced in Mongolian gerbils by ligation of a single common carotid artery (Levine, S., Payan, H., Exp. Neurol. 16: 252-255, 1966; Kahn, K., Arch. Path. 69: 544-553, 1972; Ito et al., Acta Neuropath. 32: 209-223, 1975). In our recent studies of ischemic brain edema, in gerbils, a progressive decrease in percent of dry weight (i.e., an increased water content) with a net loss of potassium and with a net gain of Na was observed in the affected hemisphere as compared to the unaffected and the control hemisphere in long-term ischemia. The present investigation has been a continuation of this study to determine the changes occurring in the brain after short period of ischemia and various recovery periods as compared to the long period of ischemia.

Methods Employed: Several groups of adult gerbils were subjected to unilateral clipping and clip release of the left common carotid artery for various periods of time. Only the gerbils with definite cerebral symptoms were selected for this study. Two different methods were used for the determination of cerebral water content: (1) wet and dry weights, and (2) specific gravity, which allows the assay of small samples of brain tissue and therefore, regional alteration in the water content can be established (Nelson, et al., J. Appl. Physiol. 30: 268-271, 1971).

Major Findings: An increase in water content of the brain tissue was observed already after 15 minutes of common carotid artery occlusion. In short-term ischemia of 1 hour duration, the cerebral water content (determined by both methods) was increased, but shows little variations until 10 hours following clip release. At this time, an increased BBB permeability to Evan's blue albumin complex was seen in the brain. A week later, the examined animals can be divided into two groups of which one shows complete recovery only. In long-term ischemia, the reduction of the specific gravity in the cortex, basal ganglia and hippocampus progressed with the length of occlusion as was previously observed by the wet and dry weight determination of water content. The contributing factors in the recovery periods such as increased blood-brain barrier permeability and tissue necrosis, which most probably are responsible for secondary increase in cerebral water content, are under evaluation.

Significance to Biomedical Research and the Program of the Institute: Cerebral edema occurs as one of the major complications of many neurological disorders such as ischemia, trauma, tumors, chemical poisoning, and others. The basic understanding of the type of edema and its development is very crucial for the clinician who is faced not only with the diagnosis, but with the appropriate selection of treatment. Thus, various investigations of this problem are essential for finding the factor or factors responsible for the occurrence of cerebral edema and its treatment.

Proposed Course of the Project: The study of brain edema in ischemia will be concerned with the continuous effort to differentiate the early cytogenic from the secondary vasogenic component of the cerebral edema. The investigation will include electron microscopic and radioisotopic evaluation of the ischemic brain in gerbils..

Publications: Go, K. G., Spatz, M., Klatzo, I., and Pappius, H. M.: The development of ischemic cerebral edema in gerbils. Proceedings of the International Symposium "Pathophysiological, Biochemical and Morphological Aspects of Cerebral Ischemia and Arterial Hypertension", Warsaw, Poland, 1976 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02001-04 LNNS
PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p>		
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Amino Acids Transport in Hypoxia, Hypercapnia and Hypocapnia</p>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: M. Spatz OTHER: T. Fujimoto I. Klatzo	Head, Section on Neurocytobiol. Visiting Fellow Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS LNNS NINCDS LNNS NINCDS
COOPERATING UNITS (if any) <p style="text-align: center;">None</p>		
LAB/BRANCH <p style="text-align: center;">Laboratory of Neuropathology and Neuroanatomical Sciences</p>		
SECTION <p style="text-align: center;">Section on Neurocytobiology</p>		
INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</p>		
TOTAL MANYEARS: <p style="text-align: center;">1.50</p>	PROFESSIONAL: <p style="text-align: center;">1.25</p>	OTHER: <p style="text-align: center;">.25</p>
SUMMARY OF WORK (200 words or less - underline keywords) <p> In severe <u>hypoxia</u>, $S_{A}O_2$ of 15-18% of the uptake of various <u>amino acids</u> tested decreased from blood to brain. <u>L-tyrosine</u>, <u>L-methionine</u> and <u>D-leucine</u> showed a lower brain uptake than <u>L-alanine</u>, <u>D-leucine</u> and <u>L-histidine</u>. However, <u>L-serine</u> was slightly affected and <u>L-glutamic acid</u> passage across the <u>blood-brain barrier</u> was not affected by hypoxia. Similar reduction of the amino acids brain uptake, except for L-leucine and L-glutamic acid, was observed in severe <u>hypercapnia</u> (pCO_2 98-150 mmHg). On the other hand, in <u>hypocapnia</u>, (pCO_2 15-18 mmHg) the brain uptake of L-tyrosine and L-methionine appeared little altered, while the other tested substances revealed a normal or increased uptake. </p>		

Project Description:

Objectives: This project is a continuation study of the effect of oxygen saturation and carbon dioxide tension on the transport of amino acids from blood to brain in rabbits.

Methods Employed: Young adult rabbits were anesthetized and ventilation controlled with a small animal respirator. By varying the rate of ventilation and concentrations of inspired gases, it was possible to regulate the pO_2 , pCO_2 and pH of arterial blood. Blood pressure was monitored throughout the experiments and blood gas levels measured in a pH/blood gas analyzer. A radioactive mixture of tritiated H_2O and a carbon-14 labeled amino acid was injected into the cerebral circulation via the right common carotid artery 15 seconds prior to decapitation. The brain was removed and a portion of cortex processed by standard techniques for radioactive counting. Uptake of the test substance was expressed in terms of a brain uptake index (BUI).

Major Findings: In severe hypoxia S_AO_2 of 15-18%, the uptake of the amino acids tested decreased from blood to brain. L-tyrosine, L-methionine and D-leucine showed a lower brain uptake than L-alanine, L-leucine, and L-histidine. However, L-serine was slightly affected and L-glutamic acid passage across the blood-brain barrier (BBB) was not affected by hypoxia. Similar reduction of the amino acids brain uptake except for L-leucine and L-glutamic acid was observed in severe hypercapnia (pCO_2 98-150 mmHg). On the other hand, in hypocapnia (pCO_2 15-18 mmHg) the BUI of L-tyrosine and L-methionine appeared little altered, while the other tested compounds revealed the BUI to be increased or normal. These changes cannot be attributed to any class of amino acid with the exception of glutamic acid, which was unaffected and belongs to acidic amino acid group, although our preliminary studies showed a difference between essential and non-essential amino acids.

Significance to Biomedical Research and the Program of the Institute: Oxygen saturation, pCO_2 and pH represent factors, which individually or combined, may play a role in altering the blood-brain barrier and contribute to the tissue damage resulting from cerebral ischemia. Since many of the amino acids are essential cerebral nutrients, the elucidation of the factors affecting their uptake of the brain should help in understanding the pathophysiological process involved in ischemia and may suggest means of preventing irreversible damage. Part of this work was presented at the International Society for Neurochemistry Satellite Symposium "Transport Phenomena in the Nervous System; Physiological and Pathological Aspects", Padua, Italy September 9-11, 1975.

Proposed Course of the Project: This model will be used for continuing evaluation of the effect of altered oxygen saturation and pCO_2 tension on the transport of other amino acids and catecholamines across the BBB. Also, blood flow studies using Xenon clearance method are planned in order to

quantitate the effect of cerebral blood flow which could be partially responsible for the observed uptake of various substances from blood to brain.

Publications: Berson, F., Spatz, M., and Klatzo, I.: Effects of oxygen saturation and $p\text{CO}_2$ on brain uptake of glucose analogues in rabbits. Stroke 6: 691-694, 1975.

Spatz, M., Berson, F., Fujimoto, T., and Klatzo, I.: Transport of Nutrients and Non-Nutrients Across the Blood-Brain Barrier in Pathological Conditions. In Cervos-Navarro, J. (Ed.): The Cerebral Vessel Wall. New York, Raven Press, 1976, pp. 225-232.

Spatz, M. and Klatzo, I.: Pathological Aspects of Brain Transport Phenomena. In Levi, G., Battistin, L., and Lajtha, A. (Eds.): Transport Phenomena in the Nervous System: Physiological and Pathological Aspects. New York, Plenum Press, 1976, pp. 479-495.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02083-03 LNNS
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PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Uptake of Glucose Analogues in Cerebellar Culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. Renkawek	Visiting Scientist	LNNS NINCDS
OTHER:	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS
	M. R. Murray	Res. Biol.	LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

1.50

PROFESSIONAL:

1.50

OTHER:

0

SUMMARY OF WORK (200 words or less - underline keywords)

The myelinated organotypic cerebellar cultures composed of glia, neurones and granular cells, but almost devoid of capillaries, took up the non-metabolizable 3-O-methyl-D-glucose (3-MG) and the partially metabolizable 2-deoxy-D-glucose (2-DG) by NaCl independent carrier mechanism in 60% and by diffusion in 40% at pH 7.0. The Km that is the apparent concentration of half maximal uptake of ^3H 3-MG and ^3H 2-DG was 11 μM and 4 μM , respectively.

Project Description:

Objectives: The changes in brain uptake of nutrients and non-nutrient substances in disease processes may depend on the integrity of the cerebral capillaries and/or neurones and glia. Since organotypic cerebellar cultures are composed of various cellular elements, we undertook to explore the possibility of utilizing organotypic CNS explants to study the permeability of brain cells to sugars in the living intact state. This study is a continued investigation of several parameters of glucose analogues uptake by cerebellar cultures in order to characterize its uptake under physiologic conditions.

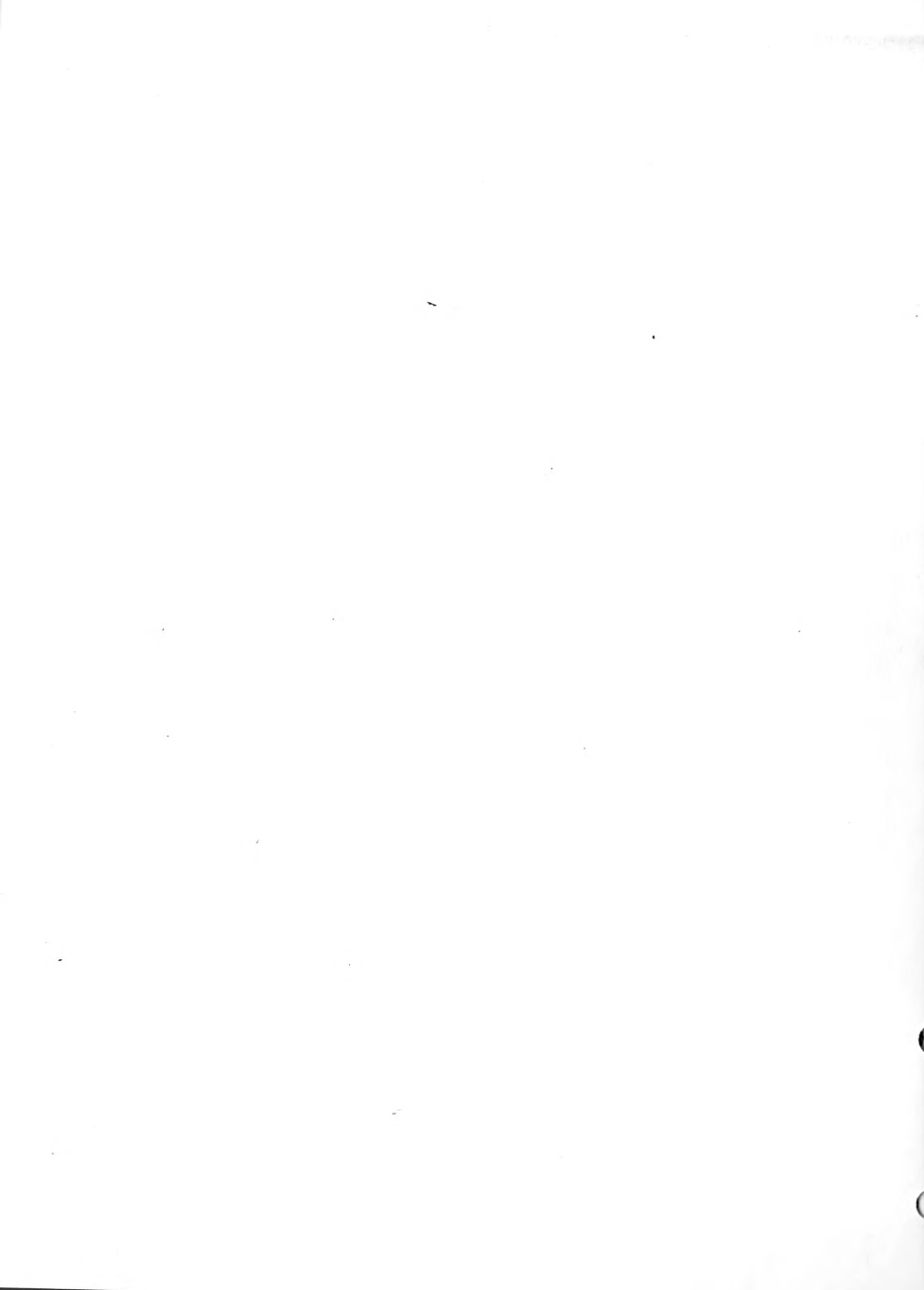
Methods Employed: The Maximow technique was used for cultivation of explants from newborn rat cerebellum. The cultures were fed twice a week with equal parts of Eagle basal media containing glutamine, fetal calf serum, bovine serum ultrafiltrate, Simm's balanced salt solution (BSS) supplemented with 10% dextrose. Fourteen day old explants, washed with dextrose-free BSS, were used for ^3H methyl-D-glucose or ^3H 2-deoxy-D-glucose (10 $\mu\text{C}/1$ ul) transport studies. The culture incubated for various periods of time contained .5 μC of the labeled substance in .5 ml dextrose-free media or BSS. For the inhibition studies glucose, methyl-D-glucose, 2-deoxy-D-glucose, xylose and phlorizin in various concentrations were added to the labeled solution. In addition, the extracellular space was determined with ^{14}C sucrose or ^{14}C inulin. After the incubation, the medium was removed and the cultures were washed three times with BSS. Pooled samples (2-7 cultures) were either weighed or assayed for protein content (Lowry, O. H.), solubilized and processed for liquid scintillation counting.

Major Findings: The myelinating organotypic cerebellar cultures were composed of glial cells, large neurones and granular cells. The highest accumulations of ^3H 3-MG and ^3H 2-DG in the cerebellar explants were found after 30 minutes of incubation at pH 7 - 7.2. At this pH, the least diffusion of the tested substances occurred from the media into the cultures. The K_m that is the apparent concentration of half maximal uptake of 3-MG and 2-DG was 11 μM and 4 μM , respectively. Elimination of NaCl from the media did not significantly change the uptake of either ^3H 3-MG or ^3H 2-DG of the explants. The uptake of ^3H 3-MG was 44 mM/mg tissue from NaCl-free medium and 42 mM/mg tissue from regular medium, while the uptake of ^3H 2-DG was 123 mM/mg ^{-1}P and 131 mM/mg ^{-1}P by the cerebellar cultures in NaCl-free and normal medium, respectively.

Significance to Biomedical Research and the Program of the Institute: The cerebellar tissue culture is an excellent model for the study of transport across the cellular membrane elements without the interference of cerebral vessels. These investigations have been focused on creating the environmental and cytogenic conditions in tissue cultures similar to the one existing in normal and diseased brain in order to determine the cellular functions in many neurological disorders associated with ischemia, edema, neurotoxicity and others.

Proposed Course of the Project: This continued investigation will be concerned with the uptake of radiolabeled glucose analogues in cultures subjected to hypoxia, and an excess of metabolites which are present in the brain tissue subjected to ischemia. This model will also be used for defining the uptake of amino acids under normal and pathological conditions.

Publications: None



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 01 NS 02084-03 LNNS
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Properties of Cerebral Capillaries in Organotypic Cultures		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: J. J. Bubis OTHER: M. Spatz M. R. Murray K. Renkawek I. Klatzo	Visiting Scientist Head, Section on Neurocytobiol. Research Biologist Visiting Scientist Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS LNNS NINCDS LNNS NINCDS LNNS NINCDS LNNS NINCDS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 1.8	PROFESSIONAL: 1.2	OTHER: 0.6
SUMMARY OF WORK (200 words or less - underline keywords) Ultrastructurally, the <u>capillaries</u> <u>cultivated</u> in the organotypic cerebellar explants showed the same characteristic <u>tight junction</u> between the endothelial cells as the one seen <u>in situ</u> . The <u>alkaline phosphatase</u> activity was confined to the plasma membrane.		

Project Description:

Objectives: The concept of blood-brain barrier (BBB) is essentially functional. Nevertheless, realization of the function to admit or exclude certain types of substances to the cerebral tissue must be based, at least in part, on morphological and histochemical factors of the capillaries. It occurred to us that the organotypic cerebellar and pia arachnoid cultures might be suitable for the isolation, identification and histochemical studies of the BBB capillaries in order to define and differentiate their function from the capillaries of tissue unrelated to the BBB system.

Methods Employed: The Maximow technique was used for cultivation of capillaries from cerebellar or lepto-meningeal explants. The cerebellum was stripped from meninges and each one sectioned into small fragments. The cerebellar tissue was placed while the meninges were spread in collagen-coated cover slips. The cultures were fed and washed twice weekly. The nutrient medium was composed of equal parts of balanced salt solution (BSS), fetal calf serum, some serum ultrafiltrate supplemented with 300 mg% of glucose. The outgrowth of cells and the formation of capillaries have been observed by light and phase microscopy for over two months. Representative cultures were photographed living, with phase and subsequently fixed in glutaraldehyde and post-fixed with osmium for plain ultrastructural examination. Another group of similar or sister cultures was incubated for alkaline phosphatase prior to the preparation of the blocks for electron microscopy.

Major Findings: This continuing investigation of enzymatic and morphologic properties of endothelial cells in cerebellar and lepto-meningeal organotypic cultures has been extended to the ultrastructural level. So far, a pilot study has been done showing the same characteristic features of the cerebellar capillaries grown in culture as in situ. The tight junctions can be identified between the endothelial cells. The activity of the alkaline phosphatase was confined to the plasma membrane.

Significance to Biomedical Research and the Program of the Institute: The cerebellar and/or lepto-meningeal organotypic cultures are a suitable model for studying the function of the endothelial cells and their role in transport and/or metabolism of the brain in health and disease. Such information is of great importance for the understanding of many neurological disease processes, especially the cerebrovascular disorders.

Proposed Course of the Project: This model will be utilized also for the study of other capillary enzymes localization in these organotypic cultures by electron microscopy. Furthermore, the cultivated microvessels will be examined by scanning microscope and their properties compared with capillaries of organs not involved in the blood-brain barrier.

Publications: Renkawek, K., Murray, M. R., Spatz, M., and Klatzo, I.: Distinctive histochemical characteristics of brain capillaries in organotypic culture. Exptl. Neurol. 50: 194-206, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02085-03 LNNS																				
PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p>																						
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Cerebrovascular Permeability to Peroxidase in Ischemia</p>																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">E. Westergaard</td> <td style="width: 40%;">Assoc. Prof. Anatomy</td> <td style="width: 20%;">Univ. Copenhagen</td> </tr> <tr> <td>OTHER:</td> <td>M. Spatz</td> <td>Head, Section on Neurocytobiol.</td> <td>LNNS NINCDS</td> </tr> <tr> <td></td> <td>K. G. Go</td> <td>Assoc. Prof. Neurosurgery</td> <td>Univ. Groningen</td> </tr> <tr> <td></td> <td>I. Klatzo</td> <td>Chief, Lab. Neuropath. Neuroanat.</td> <td>LNNS NINCDS</td> </tr> <tr> <td></td> <td></td> <td>Sci.</td> <td></td> </tr> </table>			PI:	E. Westergaard	Assoc. Prof. Anatomy	Univ. Copenhagen	OTHER:	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS		K. G. Go	Assoc. Prof. Neurosurgery	Univ. Groningen		I. Klatzo	Chief, Lab. Neuropath. Neuroanat.	LNNS NINCDS			Sci.	
PI:	E. Westergaard	Assoc. Prof. Anatomy	Univ. Copenhagen																			
OTHER:	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS																			
	K. G. Go	Assoc. Prof. Neurosurgery	Univ. Groningen																			
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat.	LNNS NINCDS																			
		Sci.																				
COOPERATING UNITS (if any) Anatomy Dept., University of Copenhagen, Copenhagen, Denmark Dept. of Neurosurgery, University of Groningen, Groningen, The Netherlands																						
LAB/BRANCH <p style="text-align: center;">Laboratory of Neuropathology and Neuroanatomical Sciences</p>																						
SECTION <p style="text-align: center;">Section on Neurocytobiology</p>																						
INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</p>																						
TOTAL MANYEARS: <p style="text-align: center;">1.75</p>	PROFESSIONAL: <p style="text-align: center;">1.00</p>	OTHER: <p style="text-align: center;">.75</p>																				
SUMMARY OF WORK (200 words or less - underline keywords) <p> <u>Mongolian gerbils</u> subjected to unilateral cerebral <u>ischemia</u> showed an increased <u>vesicular transport of peroxidase</u> in the cerebral arterioles, venules and capillaries in the affected cerebral hemisphere. The amount of transferred peroxidase across the microvessels increased with duration of cerebral blood supply deprivation, which never resulted in degeneration of the endothelial cells in the brain vessels. The intercellular spaces between the endothelial cells were free of peroxidase from the lumen to the first abluminal <u>tight junction</u>. </p>																						

Project Description:

Objectives: Cerebral infarctions occur in 30-50% of gerbils subjected to the occlusion of a single common carotid artery (Ito, U., Spatz, M., Walker, J. T., Jr., and Klatzo, I., Acta Neuropath. 32: 209-223, 1975). In the animals with cerebral injury, an augmented facilitated transport of glucose analogues was observed after 6 hours, while an increased passive diffusion of glucose occurred after 18 hours of carotid artery clipping. In gerbils without apparent cerebral damage, only the facilitated transport of glucose analogues was found to be increased irrespective of the duration of carotid artery occlusion. These findings suggested that the observed change and the type of glucose transport may depend on the integrity of the cerebral capillaries (Spatz, M., Go, K. G., and Klatzo, I., The effect of ischemia on the brain uptake of ^{14}C glucose analogues and ^{14}C sucrose. In Cervos-Navarro, J. (Ed.): Pathology of Cerebral Microcirculation. Berlin, West Germany, Walter de Gruyter & Co., 1974, pp. 361-366). Thus, the present investigation was designed to evaluate the status of the cerebral capillaries by electron microscopy in ischemic gerbils.

Methods Employed: Several groups of gerbils submitted to various periods of left common carotid artery occlusion and 1 hour release were injected with horseradish peroxidase (10 mg/100 g body weight) as the blood-brain barrier (BBB) tracer. The horseradish peroxidase was allowed to circulate for 5 minutes and thereafter the brains were perfused with para-formaldehyde. Selective tissue sections of the cerebral hemisphere were processed for light and electron microscopy from animals with and without cerebral symptoms.

Major Findings: In the post-ischemic period after 3 hours of unilateral common carotid artery occlusion, the affected cerebral hemisphere of the gerbils showed a slight increased swelling of the astroglial processes with an increased permeability of arterioles, venules and capillaries to the horseradish peroxidase tracer. These changes were more pronounced after 6 hours of ischemia, but the plasma membrane of the astroglial cells adjacent to the vessels were not permeable to the peroxidase. The occlusions of 18 hours severely damaged the plasma membranes of the neuroglial cells and the peroxidase was observed in the cytoplasm of the astroglial endfeet and in the cytoplasm of other cells. No endothelial cell damage was seen at any time, although the number of peroxidase vesicles in the endothelial cells progressively increased with the duration of ischemia. Part of this work was presented at the International Symposium on "Pathophysiological, Biochemical and Morphological Aspects of Cerebral Ischemia and Arterial Hypertension", Sept. 18-20, 1975, Warsaw, Poland.

Significance to Biomedical Research and the Program of the Institute: The elucidation of the nature and sequence of vascular and parenchymatous ischemic brain injury is of major importance in the understanding of the cerebrovascular disease process. The exploration of the established animal model system for cerebral ischemia should be helpful and useful in the comprehension and treatment of this disease process in man.

Proposed Course of the Project: This investigation has been and will be concerned with the study of ultrastructural changes in the brain during and following different periods of cerebral ischemia in order to delineate the sequential pathological processes occurring in the cerebral vessels and parenchyma.

Publications: Westergaard, E., Go, K. G., Klatzo, I., and Spatz, M.: Increased permeability of cerebral vessels to horseradish peroxidase induced by ischemia in Mongolian gerbils. Acta Neuropath., 1976 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02165-02 LNNS
PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p>		
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Uptake of Amino Acids by Pia Arachnoid</p>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: M. Spatz OTHER: M. R. Murray I. Klatzo	Head, Section on Neurocytobiol. Res. Biologist Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS LNNS NINCDS LNNS NINCDS
COOPERATING UNITS (if any) <p style="text-align: center;">None</p>		
LAB/BRANCH <p style="text-align: center;">Laboratory of Neuropathology and Neuroanatomical Sciences</p>		
SECTION <p style="text-align: center;">Section on Neurocytobiology</p>		
INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</p>		
TOTAL MANYEARS: <p style="text-align: center;">1.25</p>	PROFESSIONAL: <p style="text-align: center;">.75</p>	OTHER: <p style="text-align: center;">.50</p>
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> The comparative studies of <u>amino acids uptake</u> in <u>pia arachnoid explants</u> and <u>fetal fibroblastic</u> cultures have shown that the uptake of both the metabolizable ³H isoleucine and the non-metabolizable ³H cytoleucine take place by facilitated carrier mechanism. However, the pia arachnoid is distinctly different from the fibroblasts in being unable to accumulate the amino acid isoleucine. </p>		

Project Description:

Objectives: Facilitated carrier mediated glucose transport was described in organotypic pia arachnoid cultures recently (Spatz, M., Renkawek, K., Murray, M. R., and Klatzo, I.: Brain Res. 100: 710-715, 1975). This is a continuation study of pia arachnoid cultures in order to characterize its functions under normal and pathological conditions.

Methods Employed: Pia arachnoid explants from newborn rats were cultivated in the Maximow double cover slip depression slide assembly according to the technique of Allerand and Murray, 1968. Fetal fibroblasts were grown in MEM Eagle's media containing 1 mM glutamine and 10% fetal calf serum. Radiolabeled uptake of isoleucine and the non-metabolizable cycloleucine were studied in 14 day old cultures. The experiments were performed in triplicate in saline (BSS) washed cultures. They were incubated in .05 ml of BSS containing .5 μ c of the ^3H labeled isoleucine or cycloleucine and .025 μ c of ^{14}C inulin at pH 7.4 for 5-30 minutes. Various concentrations of either unlabeled cycloleucine or isoleucine or L-leucine or D-leucine were added to the incubation media for the inhibition studies. Thereafter, the cultures were washed, extracted with 10% trichloroacetic acid and the amount of isotope was determined by liquid scintillation counter. The protein was assayed according to Lowry et al. technique (J. Biol. Chem. 193: 265-275, 1951).

Major Findings: The isoleucine uptake of pia arachnoid explants was lower than in the fibroblastic cultures. The concentration of isoleucine in the pia arachnoid never exceeded the concentration of the medium, while the fibroblastic cultures uptake was 12 times higher than the concentration of isoleucine in incubated cultures. In both types of cultures, the uptake was saturable and 70-80% of the uptake could be inhibited by 1 mM of unlabeled isoleucine. The uptake of cycloleucine so far has been determined in pia arachnoid cultures only. Its uptake increased with time of incubation between 5-30 minutes and could be inhibited either by unlabeled cycloleucine, isoleucine, L-leucine, but not by D-leucine. The preliminary data suggest that the uptake of both the metabolizable and non-metabolizable amino acid uptake take place by facilitated carrier mediated transport in pia arachnoid, as well as fibroblasts. However, the pia arachnoid is distinctly different from the fibroblast in being unable to accumulate the amino acid isoleucine.

Significance to Biomedical Research and the Program of the Institute: The pia arachnoid explant due to its relatively simple composition of pial membrane and vessels is an excellent model for the investigation of its function. The determination and evaluations of the uptake of various substances by pia arachnoid will permit us to assess the permeability of these structures. Thus, these studies will be helpful in defining its properties and possible role in relation to blood and spinal fluid in the normal and diseased state.

Proposed Course of the Project: The continued investigations will be concerned with defining and eliciting the factors responsible for the amino acid uptake under normal conditions. Therefore, the kinetics and the influence of electrolytes, metabolites, and drugs will be determined in both types of cultures. Thereafter, this model will be used to study the amino acids uptake under pathological conditions.

Publications: Spatz, M., Renkawek, K., Murray, M. R., and Klatzo, I.: Uptake of radiolabeled glucose analogues by organotypic pia arachnoid cultures. Brain Res. 100: 710-715, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02166-02 LNNS
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Synaptosomal 2-Deoxy-D-(³ H)-Glucose Uptake In Cerebral Ischemia		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: M. Spatz OTHER: B. B. Mrsulja B. J. Mrsulja I. Klatzo	Head, Section on Neurocytobiol. Visiting Scientist Visiting Fellow Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS LNNS NINCDS LNNS NINCDS LNNS NINCDS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.0	OTHER: 0.2
SUMMARY OF WORK (200 words or less - underline keywords) <p> The <u>specific synaptosomal uptake of ³H 2-deoxy-D-glucose</u> (³H 2-DG) was progressively reduced in <u>cerebral ischemia</u> of 30-180 minutes duration. Two major groups were observed in which: (1) the specific entry of ³H 2-DG was decreased 25-53% (relative uptake 77-47%), and (2) the reduction from 45-72% (relative uptake 55-28%) as compared to the relative uptake of control (100%). After 180 minutes of ischemia, the specific uptake of ³H 2-DG was almost completely lost. The decreased synaptosomal transport function could not be restored by addition of various metabolites to the incubated media. However, complete recovery of synaptosomal ³H 2-DG uptake was observed 60 minutes after reestablishment of cerebral circulation in all gerbils subjected to 60 and 180 minutes of ischemia. </p>		

Project Description:

Objectives: Deprivation of oxygen reduces the brain uptake of glucose and glucose analogues from blood to brain in dog and rabbits, respectively (Betz, A. L., Gilboe, D. D., and Drewes, L. R.: Brain Res. 67: 307-316, 1974; Berson, F. G. Spatz, M., and Klatzo, I.: Stroke 6: 691-696, 1975). The altered glucose brain uptake could have occurred in one or more sites in which glucose transport may have taken place, such as capillary endothelium, neurons and glia. Since neurons exhibit greater oxidative metabolism than glia (Rose, S. P. R.: The Biochemistry of Neurones and Glia. In Davison, A. N. and Dobbing, J. (Eds.): Applied Neurochemistry. Philadelphia, F. A. Davis, 1968, pp. 332-355), and nerve terminals may account for a high oxidative metabolism in gray matter (Lowry, O. H. et al., J. Biol. Chem. 270: 39, 1954), it was thought to be of great importance to investigate the transport functions of synaptosomes in cerebral ischemia and post-ischemic period in gerbils.

Methods Employed: Several groups of gerbils were subjected to left common carotid artery clipping and release of the occlusion for various periods of time. The gerbils were killed by decapitation and the synaptosomes of left and right hemispheres were prepared separately by the method of Diamond and Molloy (J. Neurochem. 19: 1899, 1972). Aliquots of synaptosomal suspensions (.1 ml) were incubated in duplicate for 15 minutes at 37°C in 0.5 ml of pH 7.4 solution containing 264 mM sucrose, 26 mM potassium phosphate, and 140 mM of ^3H 2-deoxy-D-glucose (^3H 2-DG). The nonspecific entry of radioactive sugar was measured in synaptosomes in which nonradioactive glucose analogue replaced an equal concentration of sucrose in the incubation solution. The uptake was stopped by ice-cold sucrose and the contents were immediately filtered and rinsed with cold BSS (Diamond, I., and Fishman, R. A.: J. Neurochem. 20: 1533, 1973).

Major Findings: The specific uptake of ^3H 2-DG was altered by cerebral ischemia of 30-180 minutes duration. The entry of the labeled hexose was progressively decreased with the length of ischemic period, and could be divided into two major groups. The specific entry of ^3H 2-DG into ischemic synaptosomes (left) in one group was decreased 23-53% (relative uptake 77-47% and in the other group from 45-72% (relative uptake 55-28%) as compared to the relative uptake of control (right = 100%) side after 30-60 minutes of ischemia, respectively. After 180 minutes (3 hours) of ischemia, the specific entry ^3H 2-DG was almost completely lost in the synaptosomes from the ischemic cerebral hemisphere. The decreased synaptosomal transport functions of ^3H 2-DG could not be restored by addition of metabolites such as cAMP, ADP, ATP and PEP to the incubation media. However, the specific entry of ^3H 2-DG in the ischemic synaptosomes was restored after 5 minutes of reestablished cerebral circulation in two out of six animals subjected to 1 hour of ischemia and in two out of four gerbils ischemic for 3 hours. Complete recovery of ^3H 2-DG uptake by the synaptosomes from the ischemic hemisphere was observed 60 minutes after the release of the left common carotid clip in all gerbils subjected to 1 and 3 hours of occlusion.

Significance to Biomedical Research and the Program of the Institute:

The basic comprehension of neuronal function in cerebral ischemia is of major importance for: (1) understanding of the pathophysiological process occurring in the cerebrovascular disease, and (2) for selecting the best therapeutic approach to this disease.

Proposed Course of the Project: This model system will be used to study the glucose transport during the recovery period after ischemia. In addition, future investigations include the transport of amino acids and biogenic amines in the ischemic synaptosomes.

Publications: Spatz, M., Mrsulja, B. B., Mrsulja, B. J., and Klatzo, I.: Recovery of decreased synaptosomal 2-deoxy-D-(³H)-glucose uptake after cerebral ischemia in Mongolian gerbils. Brain Res. 103: 193-198, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02196-01 LNNS
PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p>		
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Isolation of Cerebral Microvessels</p>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	B. B. Mrsulja B. J. Mrsulja T. Fujimoto M. Spatz I. Klatzo	Visiting Scientist Visiting Fellow Visiting Fellow Head, Section on Neurocytobiol. Chief, Lab. Neuropath. Neuroanat. Sci.
		LNNS NINCDS LNNS NINCDS LNNS NINCDS LNNS NINCDS LNNS NINCDS
COOPERATING UNITS (if any) <p style="text-align: center;">None</p>		
LAB/BRANCH <p style="text-align: center;">Laboratory of Neuropathology and Neuroanatomical Sciences</p>		
SECTION <p style="text-align: center;">Section on Neurocytobiology</p>		
INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</p>		
TOTAL MANYEARS: <p style="text-align: center;">1.0</p>	PROFESSIONAL: <p style="text-align: center;">.75</p>	OTHER: <p style="text-align: center;">.25</p>
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> A simple method was developed to isolate <u>enzymatic</u> and <u>metabolic active fraction</u> of <u>cerebral microvessels</u> from nonvascular brain tissue which showed a saturable uptake of 2-deoxy-D-(³H)glucose. The Km for ³H 2-deoxy-D-glucose was 0.1 mM and the uptake was inhibited by the non-metabolizable 3-O-methyl-D-glucose and the inhibitors of carrier transport - phlorizin and phloretin. </p>		

Project Description:

Objectives: Few different procedures have been described for the isolation of brain capillaries recently. However, the methods were cumbersome requiring repeated use of nylon cloth sieve or glass beads columns for elimination of nonvascular cerebral tissue in order to obtain relatively pure fractions of capillaries (Goldstein, G. U., Wolinsky, J. S., and Diamond, I., J. Neurochem. 25: 715-717, 1975; Joo, F., and Karunshina, I., Cytobios 8: 41-48, 1975; and Orlowski, M., Sessa, G., and Green, J. P., Science 184: 66-68, 1974). The purpose of this study was to modify the technique for isolation of pure cerebral capillary fractions which could be used for transport and metabolic investigations of cerebral microvessels in normal and pathological conditions.

Methods Employed: Rabbit cerebral tissue (forebrain freed from pia arachnoid membrane) was homogenized in a cold Ringer's solution containing 1% bovine serum albumin at 10 mM Hepes. The homogenate was centrifuged twice at 1000 g for 10 minutes. Then the resuspended pellet was layered over a sucrose gradient and ultracentrifuged at 58,000 g for 30 minutes. The pellet was examined histologically, and histochemically and the capillary uptake of 2-deoxy-D-glucose (2-DG) was determined using radioactive hexose.

Major Findings: The isolated microvessels revealed histologically a predominance of strands, ribbons and coils of tubular structures comprised of single layer of cells devoid of smooth muscle containing reticulum fibers consistent with capillaries. Histochemically, an intense activity of γ -glutamyl-transpeptidase and butyryl-cholinesterase was demonstrated in the endothelial cells. The specific uptake of ^3H 2-DG was saturable with increased substrate concentrations. The uptake of this partially metabolizable hexose could be inhibited up to 80% with progressively increased unlabeled non-metabolizable 3-O-methyl-D-glucose, 1 mM phlorizin and 1 mM phloretin.

Significance to Biomedical Research and the Program of the Institute: The availability of an easy technique for the separation of cerebral capillaries from the nonvascular tissue provides a good method for the study of transport metabolic phenomena occurring on the blood-brain barrier level in normal and pathologic conditions, for example, in ischemia.

Proposed Course of the Project: This model system will be used for the study of glucose, amino acids and biogenic amines uptake in microvessels obtained from the brains subjected to altered pO_2 saturation, and pCO_2 tension, ischemia and recovery.

Publications: Mrsulja, B. B., Mrsulja, B. J., Fujimoto, T., Klatzo, I., and Spatz, M.: Isolation of brain capillaries: A simplified technique. Brain Res., 1976 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02197-01 LNNS
PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p>		
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Demonstration of ATPase in Cerebellar Cultures</p>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	M. Spatz K. Renkawek M. R. Murray I. Klatzo	Head, Section on Neurocytobiol. Visiting Scientist Res. Biol. Chief, Lab. Neuropath. Neuroanat. Sci.
		LNNS NINCDS LNNS NINCDS LNNS NINCDS LNNS NINCDS
COOPERATING UNITS (if any) <p style="text-align: center;">None</p>		
LAB/BRANCH <p style="text-align: center;">Laboratory of Neuropathology and Neuroanatomical Sciences</p>		
SECTION <p style="text-align: center;">Section on Neurocytobiology</p>		
INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</p>		
TOTAL MANYEARS: <p style="text-align: center;">1.0</p>	PROFESSIONAL: <p style="text-align: center;">.50</p>	OTHER: <p style="text-align: center;">.50</p>
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>Two types of ATPase activity were demonstrated in cerebellar cultures. In the glia and neuropil the enzyme activity is associated with the (Na⁺-K⁺) <u>active transport system</u>, since the enzymatic reaction was inhibited by 1 mM ouabain or showdomycin. In the neurons the ATPase reactivity was enhanced by ouabain and its function remaining to be clarified.</p>		

Project Description:

Objectives: Guth et al. (J. Histochem. Cytochem. 22: 320-326, 1974) described a new histochemical technique specific for the demonstration of ($\text{Na}^+ - \text{K}^+$) activated adenosine triphosphatase (ATPase) without the use of divalent cations such as Pb^{++} or Ca^{++} which are inhibitory to this enzyme. Therefore, the previous methods were unsatisfactory for the visualization and localization of the ($\text{Na}^+ - \text{K}^+$) ATPase. The purpose of this study was first to demonstrate the ATPase associated with active transport systems in normal organotypic cerebellar cultures in order to evaluate this enzyme activity in pathological conditions; for example, in hypoxia and anoxia in the future.

Methods Employed: The organotypic cerebellar cultures (14-28 days in vitro) and sections of cerebellum were incubated in a solution containing p-nitrophenyl phosphate substrate, KCl, MgCl, dimethyl-sulfoxide in 2-amino-2-methyl-1-propanol buffer pH 9.0 at 37°C for 1-3 hours. For the inhibition studies, 1 mM ouabain or showdomycin were added to the incubatory mixture. Control slides, cultures and frozen sections of cerebellum were incubated without p-nitrophenyl phosphate substrate.

Major Findings: The best enzyme reaction was seen in the glia cells, neurons and neuropil, but only in a few fibers after 1.5-3 hours incubation. One mM ouabain or showdomycin inhibited the enzyme reaction in the cerebellar slides and in the glia cells and neuropil, but not in the neurons of the cerebellar cultures. The enzymatic membranes reaction observed in neuronal body and processes was rather enhanced than inhibited by 1-10 mM of ouabain in the incubating solution. All the cerebellar nuclei and Purkinje cells showed a very strong reaction, while a lesser enzymatic reaction was seen in the cell membranes.

Significance to Biomedical Research and the Program of the Institute: The localization and evaluation of the ATPase in these cultures may shed more light on the function of the cellular elements of the cerebellum. Such basic information is important for the understanding of normal function of the cerebellum which could serve to elucidate the pathophysiology of neurological disorders.

Proposed Course of the Project: This investigation is incomplete yet and requires further characterization of the observed neuronal ATPase activity to elucidate its function.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02198-01 LNNS
PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p>		
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">The Effect of Hypoxia, Hypercapnia and Hypocapnia on Cerebral Capillaries</p>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: T. Fujimoto OTHER: B. J. Mrsulja B. B. Mrsulja M. Spatz I. Klatzo	Visiting Fellow Visiting Fellow Visiting Scientist Head, Section on Neurocytobiol. Chief, Lab. Neuropath. Neuroanat. , Sci.	LNNS NINCDS LNNS NINCDS LNNS NINCDS LNNS NINCDS LNNS NINCDS
COOPERATING UNITS (if any) <p style="text-align: center;">None</p>		
LAB/BRANCH <p style="text-align: center;">Laboratory of Neuropathology and Neuroanatomical Sciences</p>		
SECTION <p style="text-align: center;">Section on Neurocytobiology</p>		
INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</p>		
TOTAL MANYEARS: <p style="text-align: center;">1.25</p>	PROFESSIONAL: <p style="text-align: center;">1.0</p>	OTHER: <p style="text-align: center;">.25</p>
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> The <u>cerebral capillary fraction</u> uptake from brains of rabbits subjected to <u>pO₂ saturation</u> or <u>pCO₂ tension</u> change showed greater reduction of ³H <u>2-deoxy-D-glucose</u> uptake in hypoxia than hypercapnia and an increased uptake in hypocapnia when the uptake was calculated as percent of control uptake. The effects of the altered pO₂ saturation and pCO₂ tension in the capillary fraction were similar to the one obtained in the rabbit brain studied <u>in vivo</u> suggesting that the cerebral glucose uptake may be directly related to the capillary function. </p>		

Project Description:

Objectives: In previous studies we observed a decreased brain uptake of glucose analogues in severe hypoxia and hypercapnia, while an increased uptake of these substances was found in hypocapnia using the double isotope technique of Oldendorf (Berson, F., Spatz, M., and Klatzo, I., Stroke 7: 4, 1976). These changes in the brain uptake could be the result of an altered transport in the capillaries and/or neurons and glia. Since the isolation of metabolically active cerebral capillaries became possible (Mrsulja, B. B., Mrsulja, B. J., Fujimoto, T., Klatzo, I., and Spatz, M., Brain Res., 1976 in press) we thought that such a fraction would be of great help in clarifying certain aspects of transport processes occurring under these conditions.

Methods Employed: Rabbits were subjected to two 15 minute controlled ventilation periods: in the first 15 minutes all animals were respired to stabilize them in the normal range of blood gas values, and in the second 15 minutes the rabbits received an appropriate combination of O₂, CO₂ and N₂ for the desired stable changes in the blood gases. Thereafter, the animals were killed by decapitation and the cerebral microvessels were separated from the nonvascular tissue by fractionation (Mrsulja, B. B. et. al., Brain Res., 1976 in press). The obtained cerebral capillary fraction was used for: (1) determination of γ -glutamyl-transpeptidase and butyryl-cholinesterases, and (2) the uptake of ³H 2-deoxy-D-glucose (2-DG) was assayed in vitro.

Major Findings: The activity of both enzymes, γ -glutamyl-transpeptidase and butyryl cholinesterase, in the cerebral capillaries obtained from rabbits subjected to either hypoxia, hypercapnia or hypocapnia was not different from the one observed in control fractions. A reduction of ³H 2-DG uptake was found in the capillaries obtained from hypoxia and hypercapnia, while an increased uptake was observed in the cerebral capillary fraction from hypocapnic animals. (Relative uptake: control 100%, hypoxia 40%, hypercapnia 70%, and hypocapnia 168%.) The effect of an altered pO₂ saturation and pCO₂ tension in this study appears to be similar to that observed in vivo by the brain uptake index (BUI) technique of Oldendorf suggesting the noted decreased or increased brain uptake index of ³H 2-DG have most probably taken place on the capillary level.

Significance to Biomedical Research and the Program of the Institute: Based on our preliminary results the usefulness of the capillary fraction is promising for the study of some parameters of brain transport phenomenon occurring in both physiological and pathological conditions. The knowledge of the functional state of cerebral capillaries is extremely important, since it may either be responsible for many metabolic changes occurring in the brain, and/or it may reflect the altered metabolic state of the brain in many disease processes.

Proposed Course of the Project: This similar model will be used for the

study of the cerebral capillary function in ischemia and recovery period of gerbils subjected to cerebral ischemia.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01805-08 LNNS								
PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p>										
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Structural Changes With Membranes of Smooth Muscle Cells Under Tension</p>										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">L. Prescott</td> <td style="width: 33%;">Guest Worker</td> <td style="width: 33%;">LNNS NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>M. W. Brightman</td> <td>Head, Sect. on Neurocytol.</td> <td>LNNS NINCDS</td> </tr> </table>			PI:	L. Prescott	Guest Worker	LNNS NINCDS	OTHER:	M. W. Brightman	Head, Sect. on Neurocytol.	LNNS NINCDS
PI:	L. Prescott	Guest Worker	LNNS NINCDS							
OTHER:	M. W. Brightman	Head, Sect. on Neurocytol.	LNNS NINCDS							
COOPERATING UNITS (if any) <p style="text-align: center;">None</p>										
LAB/BRANCH <p style="text-align: center;">Laboratory of Neuropathology and Neuroanatomical Sciences</p>										
SECTION <p style="text-align: center;">Section on Neurocytology</p>										
INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</p>										
TOTAL MANYEARS: <p style="text-align: center;">0.9</p>	PROFESSIONAL: <p style="text-align: center;">0.6</p>	OTHER: <p style="text-align: center;">0.3</p>								
SUMMARY OF WORK (200 words or less - underline keywords) <p> In order to ascertain the effects of <u>stretching</u> on internal structure of <u>smooth muscle cell membranes</u>, the nerve connectives of the mollusk <u>Aplysia</u> were stretched twice and three times their normal length, fixed, and either <u>freeze-fractured</u> or embedded in plastic. The connectives contain numerous, isolated smooth muscle cells (SMC). The sarcolemma of the SMC, like that of vertebrate smooth muscle, is indented in the form of numerous <u>pits</u> or caveolae. Unlike most other species, the pits leave a natural marker in the form of "bumps" about 65-130 Å wide inside their membranes. In the stretched connectives, the fractured sarcolemmas display many more clusters of these "bumps", indicating that the caveolar membrane had become <u>flattened</u> and <u>pulled into the fracture plane</u>. The pits do not appear to be involved in pinocytosis; even after prolonged soaking of the connective in peroxidase solutions; the only layer of reaction product was that bound to the pits which communicated with the extracellular space. The results signify that the pits can be unfolded during stretch and thus increase the extensibility of the sarcolemma. Because of its structure, the pit membrane should be distinguishable from isolated fragments of sarcolemma and so lends itself to biochemical assessment of a possible role in stimulus - contraction coupling. </p>										

Project Description:

Objectives: To examine changes in structure of sarcolemmal caveolae which have hitherto been considered as pinocytotic.

Methods Employed: Ganglia in the mollusk *Aplysia*, are surrounded by a sheath in which is embedded isolated, separate smooth muscle cells. The sheaths were either quenched in liquid nitrogen or fixed first before freezing. Bits of sheath were then fractured in vacuo. Other sheaths, stretched three times their normal length were also fixed, frozen, and cleaved. Portions of all sheaths were embedded in plastic and sectioned for electron microscopy, including several that had been soaked in HRP for 30 minutes to 6 hours and one specimen in 90 μ M CaCl_2 .

Major Findings: The sarcolemma, like that of vertebrate smooth muscle bears hemi-desmosomes and is indented to form many pits or caveolae. Unlike most other species, the pits have a natural marker in the form of "bumps", about 65-130 Å wide, within their membranes. In the stretched connectives, the fractured sarcolemmas display many more clusters of these "bumps" indicating that the caveolar membrane had become flattened and pulled into the fracture plane. The muscle cells were thinner in the stretched preparations and the sarcolemma of some were broken. HRP coated the pits, but did not appear to have been pinocytosed.

Significance to Biomedical Research and the Program of the Institute: The sarcolemmal pits can be stretched and so provide for extensibility of the muscle membrane. Because of their distinctive structure, their physical and chemical properties can be compared with the rest of the sarcolemma and may include a leak in excitation-contraction coupling.

Proposed Course of the Project: An account of this work is to be published.

Publications: Prescott, L., and Brightman, M. W.: Distinctive particles within the frozen, cleaved membrane of caveolae in *Aplysia* smooth muscle. Tissue & Cell, 1976 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02086-03 LNNS
PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p>		
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Regeneration in Vertebrate and Invertebrate Nerves</p>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: R. Shivers OTHER: M. W. Brightman	Guest Worker Head, Sect. on Neurocytol.	LNNS NINCDS LNNS NINCDS
COOPERATING UNITS (if any) <p style="text-align: center;">None</p>		
LAB/BRANCH <p style="text-align: center;">Laboratory of Neuropathology and Neuroanatomical Sciences</p>		
SECTION <p style="text-align: center;">Section on Neurocytology</p>		
INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</p>		
TOTAL MANYEARS: <p style="text-align: center;">1.5</p>	PROFESSIONAL: <p style="text-align: center;">1.2</p>	OTHER: <p style="text-align: center;">0.3</p>
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> In order to determine the structural changes <u>inside the membranes of nerves</u> <u>and surrounding glia during degeneration and regeneration</u>, the ventral <u>nerve</u> <u>cord of crayfish</u> was chosen because of its accessibility, and therefore, minimal scarring after the cord is sectioned. In intact animals and in those with sectioned roots, the nerve roots were either soaked in peroxidase (HRP) or <u>freeze-fractured</u>. In both normal and degenerating roots, a hitherto unsus- pected system of <u>transglial channels</u> accessible to HRP has been recognized in both thin plastic sections and freeze-fracture replicas. The channels, about 240 Å wide, are straight and short, being as long as the thin glial sheets are wide. The frequency is about 16 per μm^2 in both normal and degenerating areas. In replicas, the channel openings appear as circular depressions or protuber- ances. In degenerating roots, the glial cells become disorganized but still lie close to axons. The <u>gap junctions</u> between glial cells increase in number but diminish in size. The observations signify that in the ventral cord, trans- glial channels may provide extracellular short-cuts whereby water, ions and metabolites move quickly from interstitial fluid to axonal membranes. The observations raise the question of whether shortened diffusion pathways per- meate glial scars in mammals. </p>		

Project Description:

Objectives: To examine structural changes inside the membranes of nerves and surrounding glial cells during degeneration and regeneration.

Methods Employed: The nerve roots of the 6th abdominal ganglion in crayfish are so superficial that only a little dissection is required for their exposure. Scarring is, therefore, minimal. Exposed roots were soaked in peroxidase for 1 hour. In other crayfish intact roots and those that had been cut 1 to 5 weeks prior to fixation were frozen and fractured.

Major Findings: In the normal and regenerating nerve roots of crayfish, a hitherto unsuspected system of transglial channels accessible to peroxidase has been recognized in both thin sections and freeze-fracture replicas. The channels, about 240 Å wide, are straight and short, being, as long as the thin glial sheets are wide. Then frequency is about 16 per μm^2 normal roots and 13 per μm^2 in regenerating area. In replicas, the channel openings appear as circular depressions or pits and protuberances or bosses.

In regenerating roots, the glial cells become disorganized but still lie close to axons. The gap junctions between glial cells increase in number but diminish in size.

Significance to Biomedical Research and the Program of the Institute: The diffusion path from periglial fluid spaces to axonal membrane is shortened by transglial channels which could act as short-cuts for the flow of metabolites and ions.

Proposed Course of the Project: The work on transglial channels is being prepared for publication. The observations on glial junctions are to be extended to longer time periods of regeneration.

Publications: Brightman, M. W., Shivers, R. R., and Prescott, L.: Morphology of the walls around fluid compartments in nervous tissue. In Cserr, H. (Ed.): Fluid Environment of the Brain. New York, Academic Press, 1975, pp. 3-29.

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Effects of Hypertension on the Permeability of Cerebral Endothelium to Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. S. Robinson	Guest Worker	LNNS NINCDS
OTHER:	M. W. Brightman	Head, Sect. on Neurocytol.	LNNS NINCDS
	S. I. Rapoport	Neurophysiologist	LNP NIMH

COOPERATING UNITS (if any)

Laboratory of Neurophysiology, NIMH

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

1.2

PROFESSIONAL:

1.1

OTHER:

0.1

SUMMARY OF WORK (200 words or less - underline keywords)

Does an increase in intraluminal pressure of cerebral blood vessels lower the blood-brain barrier to protein? The arterial blood pressure of rats was recorded continuously from the femoral artery through a pressure gauge. After the vasopressor, Aramine, was injected intravenously, the pressure rose from 90 to 150 mm Hg. At this threshold, blood-borne peroxidase escaped from some blood vessels and appeared as brown spots in fixed, incubated sections. The entire brain was sectioned and the brown spots tallied. There was a gradient of exudate numbers, the highest being in the forebrain, the lowest in the brain stem. Further, the total number of openings in immature rats was less than in mature animals. When Evans blue dye was given directly before Aramine and HRP sometime later, only blue spots appeared 1 to 2 hours after Aramine infusion; the barrier opening was reversible. When the intraluminal pressure was raised by rapid infusion of a bolus of saline or aldehyde, into one carotid artery, many brown exudates appeared on the injected side within a few minutes. The results indicate that the vascular infusion of a bolus of fluid, including radiocontrast material, may result in a transient break in the blood-brain barrier to proteins.

Project Description:

Objectives: To see if and how a rise in the intraluminal pressure of cerebral blood vessels affects their permeability to blood-borne protein.

Methods Employed: The baseline arterial pressure is recorded continuously from the femoral artery of adult rats. A solution of Evans blue, then peroxidase (HRP) is injected into a femoral vein followed by 0.5 mg/kilo body weight of the vasopressor, Aramine. When the blood pressure (bp) exceeds 60 mg Hg above resting level, the brain is fixed and processed for HRP activity. The HRP exudates appear as brown spots which are counted for each of five regions of the brain. In a second group, the blue dye is given first and from 2 minutes to 20 hours after Aramine, HRP is given to test for reversibility of barrier opening. In a third group, a bolus of saline or fixative with HRP is rapidly infused into one carotid artery.

Major Findings: In 30 rats, the rise in bp during Aramine injections was accompanied by the appearance of randomly scattered blue spots in the cortex and in the parenchyma and many more brown, HRP spot-exudates. The number of spots is greatest in the cerebrum and least in the medulla. The opening of the barrier is reversed within 1 to 2 hours. In 12 rats given the fluid bolus, the number of HRP exudates were far more numerous but their distribution was similar on the side of the brain supplied by the bolus-injected carotid artery.

Significance to Biomedical Research and the Program of the Institute: A moderate, transient hypertension is accompanied by an escape from cerebral blood vessels of blood-borne substances as large as albumin, HRP and presumably, smaller substances. Thus, therapeutic or toxic agents, normally excluded from the brain, can enter during such a brief episode.

Proposed Course of the Project: To publish these findings.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02145-02 LNNS						
PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p>								
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Identification of Neurons Having Terminals in the Median Eminence and Area Postrema</p>								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT								
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: R. D. Broadwell</td> <td style="width: 33%;">Staff Fellow</td> <td style="width: 33%;">LNNS NINCDS</td> </tr> <tr> <td>OTHER: M. W. Brightman</td> <td>Head, Section on Neurocytol.</td> <td>LNNS NINCDS</td> </tr> </table>			PI: R. D. Broadwell	Staff Fellow	LNNS NINCDS	OTHER: M. W. Brightman	Head, Section on Neurocytol.	LNNS NINCDS
PI: R. D. Broadwell	Staff Fellow	LNNS NINCDS						
OTHER: M. W. Brightman	Head, Section on Neurocytol.	LNNS NINCDS						
COOPERATING UNITS (if any) <p style="text-align: center;">None</p>								
LAB/BRANCH <p style="text-align: center;">Laboratory of Neuropathology and Neuroanatomical Sciences</p>								
SECTION <p style="text-align: center;">Section on Neurocytology</p>								
INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</p>								
TOTAL MANYEARS: <p style="text-align: center;">1.0</p>	PROFESSIONAL: <p style="text-align: center;">1.0</p>	OTHER: <p style="text-align: center;">0</p>						
SUMMARY OF WORK (200 words or less - underline keywords) <p>Protein, such as peroxidase (HRP) circulating in the blood, cannot reach neurons from surrounding capillaries because of the blood-brain barrier. We have found, however, that the <u>protein can enter motor, sensory and preganglionic neuronal cell bodies</u> by moving retrogradely up their axons after crossing permeable capillaries in muscle and certain brain regions. Thus, high concentrations of HRP injected intravenously into mice cross muscle capillaries and are pinocytosed by axon terminals at myoneural junctions. The protein, then transported within axons, reaches the cell bodies of cranial motor neurons such as VI, VII, IX, X, XI and XII. Since the XII nerve nucleus lies near permeable cerebral vessels, it is possible that their cell bodies may incorporate HRP directly from extracellular clefts. The entry of HRP is from the periphery, however, because when one <u>hypoglossal</u> nerve was ligated, its cell bodies were unlabeled, whereas, those on the opposite side were. <u>Ligation or crush increases lysosomal activity</u> so that HRP, already delivered to cell bodies, is enzymatically degraded. The results imply that protein, including nerve growth factor, and tetanus toxin and other macromolecules can enter neurons from peripheral blood long after the substance has been cleared from the blood.</p>								

Project Description:

Objectives: To follow the uptake and retrograde transport of blood-borne protein in various neurons.

Methods Employed: Peroxidase (HRP), from 30-90 mg, is injected intravenously in intact mice and in those with one hypoglossal nerve ligated. In other mice, the HRP is perfused through the cerebral ventricles. The brains and nerves are examined by light- and electron-microscopy.

Major Findings: HRP, by crossing permeable vessels of muscle and autonomic ganglia, becomes available to axon terminals which pinocytose the protein. The HRP is transported retrogradely in their axons to the parent cell bodies within the central nervous system (CNS). Ligation of one XII nerve not only prevents this movement, but when HRP is allowed to reach the cell bodies before ligation, the protein is enzymatically degraded at a far greater rate than within intact neurons. HRP, coming from the ventricles, is pinocytosed to a lesser degree by neuronal cell bodies directly and enters their lysosomes. It appears that, with respect to retrograde transport, labeling of smooth endoplasmic reticulum is more certainly indicative of HRP transport to cell bodies than is entry into lysosomes.

Significance to Biomedical Research and the Program of the Institute: Blood-borne protein is taken up by axon terminals of certain neurons projecting within and without the CNS and transported back to their cell bodies in brain and spinal cord. In this way, other proteins such as tetanus toxin, nerve-growth factor and viruses may also bypass the blood-brain barrier to enter the CNS long after they have been cleared from the blood.

Proposed Course of the Project: To determine, electronmicroscopically, in which organelles the HRP travels within axons, to see whether they accumulate on either side of a ligature, and to compare this mode of transport with that in neurosecretory neurons.

Publications: Brightman, M. W., Prescott, L., and Reese, T. S.: Inter-cellular junctions of special ependyma. In Scott, D. E., and Kobayashi, M. (Eds.): Proceedings of the Second International Symposium on Brain-Endocrine Interaction, Basel, Switzerland, S. Karger, 1975, pp. 146-165.

Brightman, M. W., and Reese, T. S.: Membrane specializations of ependymal cells and astrocytes. In Tower, D. B. (Ed.): The Nervous System, Vol I: The Basic Neurosciences. New York, Raven Press, 1975, pp. 267-277.

Broadwell, R. D., and Brightman, M. W. : Entry of peroxidase into neurons of the central and peripheral nervous systems from extracerebral and cerebral blood. J. Comp. Neurol. 166: 259-283, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02200-01 LNNS								
PERIOD COVERED July 1, 1975 to June 30, 1976										
TITLE OF PROJECT (80 characters or less) Freeze-Fracture of Cell Membranes Intercalated With Lipids										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT										
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">M. Sato</td> <td style="width: 33%;">Visiting Fellow</td> <td style="width: 33%;">LNNS NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>M. W. Brightman</td> <td>Head, Sect. on Neurocytology</td> <td>LNNS NINCDS</td> </tr> </table>			PI:	M. Sato	Visiting Fellow	LNNS NINCDS	OTHER:	M. W. Brightman	Head, Sect. on Neurocytology	LNNS NINCDS
PI:	M. Sato	Visiting Fellow	LNNS NINCDS							
OTHER:	M. W. Brightman	Head, Sect. on Neurocytology	LNNS NINCDS							
COOPERATING UNITS (if any) None										
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences										
SECTION Section on Neurocytology										
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014										
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:								
1.5	1.2	0.3								
SUMMARY OF WORK (200 words or less - underline keywords)										
<p> To test the <u>hypothesis</u> that, in freeze-fractured <u>membranes</u>, the <u>non-particulate phase is lipid</u>, attempts are being made to <u>intercalate lipid</u> into cell membranes. If the density of particles, presumably protein, in a unit area decreases, then the thesis is correct. Large amounts of <u>fatty acids (fa)</u>, injected into a carotid artery of rats, acts as a detergent and rapidly crosses vessels. Droplets of the polyunsaturated fa, linolenic acid, are osmiophilic, and can be seen fusing with the endothelial cell membrane. Droplets then cross the sarcolemma of the <u>smooth muscle cells (SMC)</u> of the tunica media. The fa droplets in the SMC tend to cluster around <u>mitochondria</u>, whose oxidative phosphorylation is known to be uncoupled by fa. No droplets were ever seen within the nucleus of any cell type. Since the <u>choroid plexus</u> epithelium has numerous particles within its cell membrane, fa was also perfused throughout the cerebral ventricles. Droplets not only entered these cells but passed extracellularly between them. Neutral lipids, such as <u>cholesterol</u>, are being infused for 48 hours or fed for 3 months in order to determine whether they can be intercalated more readily into stroke-prone rats as compared with normal animals. <u>Liposomes</u> are also being applied to cells <u>in vivo</u> and <u>in vitro</u>. </p>										

Project Description:

Objectives: To determine whether particles within frozen, cleaved cell membranes can be redistributed by intercalating lipid into the membranes and whether this effect is more pronounced in stroke-prone rats.

Methods Employed: Polyunsaturated fatty acids (fa), mono- and di-glycerides are infused either into the carotid artery of adult normal and stroke-prone rats or into the stomach of 7 day old rats. A high cholesterol diet is fed to others for several months or infused into the cerebral ventricles. Liposomes are to be incubated with choroid plexus in vitro. Tissue is embedded in plastic or freeze-fractured and viewed by light and electron optics.

Major Findings: Droplets of linolenic acid act as a soap and freely cross cell membranes. Droplets have been "caught in the act" of fusing with endothelial cell membranes and penetrating into the cytoplasm. In smooth muscle, fa preferentially clusters around mitochondria. Although the perinuclear cistern of various cell types is penetrated, the nucleus is always free of droplets. The fracture faces of choroid plexus epithelium is studded with numerous particles believed to be protein. Prolonged cholesterol feeding does not change the distribution of the particles.

Significance to Biomedical Research and the Program of the Institute: Because of its high content of protein particles, the cell membrane of choroid plexus epithelium and, to a lesser extent, blood vessels, are well suited to test the hypothesis that the non-particulate phase in fracture planes is lipid.

Proposed Course of the Project: To incubate choroid plexus with liposomes composed of bileaflets for 1 minute to 48 hours in order to see whether particle-free patches appear within the membranes as a result of fusion.

Publications: None

ANNUAL REPORT

July 1, 1975 through June 30, 1976

Laboratory of Neural Control, Intramural Research

National Institute of Neurological and Communicative Disorders and Stroke

Robert E. Burke, M.D., Chief (Acting)

Introduction

From its inception in 1967 until April, 1975, the Laboratory of Neural Control (LNLC) has as its primary mission the development of methods and strategies for applying the results of fundamental neuroscience research to the problem of prosthetic aids for neurologically handicapped patients. The founding Chief of LNLC recruited a staff with multidisciplinary interests, including the fields of bioengineering, physics, mathematics and computer sciences in addition to training in neurophysiology and/or in medicine. There have been no identifiable boundaries between applied and fundamental research interests within the Laboratory. Problems of prosthetic devices have suggested research questions of basic importance and a number of technical developments arising from the prosthetics area have opened new possibilities for experiments. Because of the converse dependence of applied studies on the results of fundamental research, the Laboratory has always had a strong program of basic investigations. The transfer of management of the Neural Prosthesis Program to the Fundamental Neurosciences Program, and the change in emphasis as to the description of current research work in LNLC do not signal any real alteration in the close mixing of "fundamental" and "applied" studies which has characterized this Laboratory from its beginning. Members of the Fundamental Neurosciences Program have maintained close contact with members of LNLC in order to facilitate the interchange of ideas which has proven so fruitful in the past.

Present Organization

Essentially all of the research work currently in progress in LNLC has to do with investigation of central nervous system mechanisms involved in the control of movement in mammals. Several projects are concerned with analysis of movement-related systems in the spinal cord, studied mainly in the cat. Such projects include detailed studies of the motor unit population of a typical large limb muscle in the cat, the medial gastrocnemius (Intrinsic Properties of Motor Units), physiological and anatomical studies of neuronal circuits in

the cat spinal cord as they relate to the motoneuron output cells (Motor Control Systems in the Spinal Cord), and studies designed to provide information about activity in spinal neurons and in primary afferent fibers during locomotion in awake, intact cats (Neuron Activity During Locomotion). These aspects of motor control as studied at the spinal cord level are clearly interrelated, and there is a corresponding overlap of personnel engaged in the projects.

Another long range project in LNLC has to do with studies of the activity of neurons in the monkey motor cortex during voluntary limb movements, either spontaneous or conditioned. (Cortical Mechanisms of Voluntary Motor Control). This project also has a close interrelation with the others noted above, since the inferences drawn on the basis of observations of correlations between cortical neuron activity and limb movement depend very much on the interaction of descending cortical activity with spinal segmental mechanisms. This latter subject is as yet not well defined and remains an important next step in motor control research.

Some of the research projects mentioned above utilize methods and techniques that have become standard in neurophysiological laboratories. However, very important aspects of the current work on cortical voluntary control of movement and of neuronal activity during locomotion in intact cats require the development of novel methods for obtaining useful recordings from experimental animals that are awake, comfortable and behaving with minimal restraint. Such developments have been possible within LNLC because the Laboratory is suitably equipped and, more importantly, has staff members with the interest and expertise to use new materials and novel designs for electrodes to solve the problems inherent in chronic recording from moving animals. The research project "Techniques for Making Connections with the Nervous System" is maintained as a separate, identifiable item to highlight the importance of applied technology to furthering the research goals of the Laboratory. The work done under this project on materials testing, fabrication techniques, etc., is almost never done as an end in itself, but rather in relation to a specific problem in one of the Laboratory research projects. Nevertheless, the findings and methods developed in the area of applied technology have proven useful to other investigators outside the Laboratory, both at NIH and in the academic community throughout the country.

The field of motor system neurophysiology has in the past advanced mainly through experimental approaches using anesthetized or neurologically reduced animal preparations suitable for the available recording techniques. Much remains to be done with such preparations but the field has clearly advanced to a point where

meaningful questions must be asked in preparations that are in fact moving normally. LNLG is uniquely equipped to carry forward research in this direction. Much of the work with moving animals has been, and will necessarily remain, in the area of developing the necessary tools to produce meaningful data. However, this research is reaching the stage at which questions of fundamental physiological importance can be answered, applying the wealth of information from reduced preparations to the problem of normal animal movement.

Project Summaries

Motor Control Systems in the Spinal Cord.

This project involves a neurophysiological and, to some extent, neuroanatomical study of the neuronal circuits that are present in spinal cord segments and are related to the control of movement. Over the past several years, we have defined some quantitative patterns of synaptic organization among motoneurons of the medial gastrocnemius (MG) pool which innervate defined types of motor units. We have been concerned with this motor unit pool because of the growing evidence that the different types of motor units present in this muscle reflect functional specializations optimized for different ranges of motor output. Further work along this line requires analysis of interneuron circuits in the cord segment, with particular reference to the convergence of various sorts of primary afferent input to particular interneuron pathways, and the control of transmission along these pathways by other inputs descending to the cord from supraspinal structures. Information about patterns of input convergence can be obtained from observation of changes in synaptic potentials within alpha motoneurons produced by activation of one or another input system, and of both systems together at appropriate intervals.

The series of experiments completed this year on firing patterns of MG motoneurons after cutaneous nerve stimulation has been done largely to provide evidence that the recruitment patterns of alpha motoneurons within a given motor nucleus are controlled importantly, and perhaps overwhelmingly, by factors that can be described as related to "synaptic organization". There is in the literature an often expressed assumption that recruitment of motoneurons depends only on some property intrinsic to the cells themselves, such as neuronal size. On closer examination of the available data from this and other laboratories, such an explanation appears highly unlikely. Our present demonstration that large changes in recruitment pattern can occur in response to a single input system seems very

strong evidence in favor of the view that motoneuron "excitability" and the order of recruitment are properties of the spinal segment system, i.e., are due to the way in which synaptic input is organized to particular neurons in the motor pool, and to the interaction of such organizational factors with intrinsic motoneuron properties such as input resistance, time constant, and absolute voltage threshold.

We have initiated this year a series of experiments designed to study individual spinal interneurons that project directly to alpha motoneurons. As a first step, a mapping of candidate "last-order" interneurons was done using retrograde transport of the protein marker, horseradish peroxidase. These maps can now be used to guide a search for individual interneurons, recorded mainly with extracellular techniques, that can be shown to project monosynaptically to motoneurons recorded with a second micropipette using the technique of spike triggered averaging. This will be a technically difficult study, but it seems worth the effort since we may be able to gain information about the organization of segmental interneuronal circuits not obtainable in any other way. Secondly, the method may provide an opportunity to examine the details of synaptic transmission between interneurons and motoneurons, resulting in data that can be compared with results from group Ia to motoneuron synapses. The latter results are at present controversial and difficult to interpret, and comparison with other synaptic systems to motoneurons should be helpful.

Intrinsic Properties of Motor Units.

This project is an outgrowth of the above project on Spinal Motor Control Systems. It was identified as a separate item in last year's Annual Report because recent work in this area has taken a tack toward examination of factors that either maintain motor unit characteristics or produce changes in them. Such factors are often referred to as "trophic", but it must be noted that the current state of information about "trophic" interactions between motoneurons and the muscle fibers they innervate is filled with confusing and sometimes contradictory evidence. Much of the experimental work in this field has used whole muscles that are in fact not pure populations of one muscle fiber (or motor unit) type. Data accumulated on the basis of observations of whole muscle contractions, or whole muscle biochemistry, are often difficult to interpret because of the heterogeneity of the fiber populations tested. We have been convinced that an attack on some issues of trophic interactions must be attempted by analyzing the properties of motor units one at a time. This is a painstaking and difficult approach but it has two advantages: 1.) some of the difficulties with mixed unit populations can be resolved

without ambiguity; and 2.) possible alterations in electrophysiological properties of alpha motoneurons and their synaptic inputs can also be tested.

This experimental approach is possible in LNLN because we have accumulated a detailed data base on the normal characteristics of motor units in the MG muscle of cats. Our experience with analyses of MG motor unit populations after chronic immobilization or compensatory hypertrophy indicate that the method is valid and provides, when combined with muscle histochemistry, a rather complete picture of altered motor unit populations. It is particularly interesting that there is preliminary evidence suggesting a change in synaptic strength with immobilization atrophy, our first indication so far of trophic effects of altered use on CNS synaptic transmission.

Part of this year's program has included a detailed analysis of the anatomy of the medial gastrocnemius (MG) and soleus motor nuclei using peroxidase tracer techniques. The results have confirmed previous less direct evidence as to the distribution of alpha and gamma motoneurons within the motor nuclei, and has also provided new data on tracer uptake characteristics of these two species of motoneurons. Anatomical cell marking techniques will be added to our technical armamentarium in further work in this area.

Neuron Activity During Locomotion.

This project represents the coming together of several lines of work within LNLN over the past 5 years. We have been concerned about the general problem of how to apply information gained from traditional neurophysiological experiments (studies of spinal reflexes, properties of peripheral receptors, etc.) to studies of CNS control of normal movements in intact animals. Past Annual Reports have mentioned efforts to record activity in individual motor units during normal movements using methods permitting motor unit type identification. This approach, although frustrated by severe technical problems, has led to a focus on the cat hindlimb and on treadmill locomotion as a model system well suited for investigation.

Over the past several years, experience with various types of implanted electrodes for recording unitary neuronal activity in intact moving animals has shown that some methods are indeed suitable for obtaining data on the patterns of activity in peripheral afferent fibers. The major problem at the moment is to obtain reasonably large samples of data from afferents that can be identified unambiguously as to specific type and receptive field. Our experience to date has been sufficiently encouraging to justify assembling a sophisticated

data recording and processing system including a videotape capability to record movements on the treadmill, a magnetic tape and computer system to take electrophysiological data, and a time code generator to permit movement and unit data to be correlated.

Recording of identifiable afferents during locomotion is a suitable first step in this work, since there is very little information about activity in any type of peripheral afferent during normal walking in intact cats. Such data is particularly important with respect to joint receptors and to muscle spindle afferents, since, in the latter case, the degree of alpha-gamma coactivation during locomotion is a point on which there is some present controversy.

Cortical Mechanisms of Voluntary Motor Control.

Over the past decade, there has been considerable interest in recording the behavior of neurons in the monkey motor cortex during the production of voluntary movements. Such experiments were made possible by the development of appropriate recording techniques in the Laboratories of NIMH. However, the experimental design most widely used leaves a number of important issues unexplored, including the specificity of the association between neuron activity and particular movements, and the destination of the cortical cell axon (i.e., it is usually unknown whether the axon reaches the spinal cord or ends in the brain stem, subcortical nuclei or even within the cortex itself).

The present project was begun a number of years ago mainly to examine the feasibility of using the discharge patterns of motor cortex neurons to control external (prosthetic) devices. This goal led to the development of electrodes that can be implanted chronically in motor cortex to record the activity of individual neurons over many hours or even days. Secondly, the goal also dictated exploration of the issue of whether monkeys could be trained to produce various rates of cortical cell firing irrespective of whether or not movements were produced, or of the specific nature of movements emitted. These original goals have now led to the development of an experimental capability in which a number of fundamentally important questions can be attacked, including the issue of neuron - movement specificity and the identification of axonal destination of recorded cortical neurons.

Monkeys, trained to alter cortical cell firing rates to get rewards, manage this situation by producing movements of various sorts. Cortical neurons without correlation to any movement are not successfully used to attain rewards. Thus, the monkeys are "learning", not to produce cell discharge in isolation, but rather to produce a movement associated with the discharge of the cell being recorded. The movement

is not selected by the experimenter. Monkeys in this situation, working with a particular cell over the long time periods made possible by the map pin electrodes, appear to refine their movements to just those appropriate to the neurons under test. Thus, the answer to the question of cell-movement specificity can be effectively sought using this experimental design.

The map pin electrodes can be chronically implanted in the spinal cord. It seems entirely feasible that these can be used to stimulate axons of cortical neurons arriving at the cervical spinal cord and thus identify at least that subset of recorded neurons that are indeed corticospinal.

Models of Neural Interaction.

The main problem dealt with during the past year has been a theoretical analysis of some possible models for signal processing in neuronal networks. Such analyses are relevant to information processing anywhere in the CNS, and it would be a significant advance if the kinds of conceptual network models that have been developed for processing spatially organized input such as in the visual system can be adapted for dealing with the complex spatial - temporal coding patterns evidently present in input from somatic proprioceptors.

Techniques for Making Contact with the Nervous System.

This project is in some respects not a formal research project in the sense that the term is used for other aspects of the LNLC program. Many of the activities relate to techniques and instrumentation developed to fill specific needs in the other LNLC projects. Such developments include further modification of the already very successful map pin electrode, now in first trials for implantation into the spinal cord. An implantable force transducer to sense individual muscle forces during normal movements appears successful and should greatly benefit our kinesiological work in locomotion. Special instrumentation, such as a miniaturized 10 channel high impedance amplifier and a servo-controlled muscle stretching device, are precisely tailored for LNLC projects but may well find wider use outside the Laboratory in time.

Other aspects of this project relate to collaborative work with research groups outside LNLC, but heavily dependent on the technical expertise available within this Laboratory. Examples include the final development of a floating microdrive device, built in the Biomedical Engineering and Instrumentation Branch primarily for use in clinical research in epilepsy, but constructed, tested and modified with significant input from LNLC staff. A 16 channel EEG telemetry system has been assembled and modified for patient monitoring

in the Clinical Center again with a large input from LNLC staff members. Another very promising collaboration has been in adapting the polymer, Parylene-C, for use as a substrate for tissue culture. LNLC is almost unique in its Parylene facility and this facility has been used to further the tissue culture research on nerve and muscle cells being done in the Behavioral Biology Branch, NICHD.

Finally, it should be noted that some of the work done in this Project is in fact self-contained research. This has been mainly evaluations of various materials (conductors, insulators, etc.) for suitability for use in further development of specialized techniques. Such in-house testing is not particularly glamorous but is necessary for the efficient and cost-effective development of novel methodologies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01686 08 LNLCL
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) MOTOR CONTROL SYSTEMS IN THE SPINAL CORD		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <div style="display: flex; justify-content: space-between;"> <div> Principal Investigator: Robert E. Burke, M.D. Other Investigators: Kenro Kanda, M.D., Ph.D., Bruce Walmsley, Ph.D., Peter Strick, Ph.D., C.C. Kim, Ph.D. </div> <div> Acting Chief, LNLCL,NINCDS Visiting Fellow, LNLCL,NINCDS Visiting Fellow, LNLCL,NINCDS Staff Fellow, LNP,NIMH Visiting Fellow, LNP,NIMH </div> </div>		
COOPERATING UNITS (if any) Laboratory of Neurophysiology, NIMH		
LAB/BRANCH Laboratory of Neural Control SECTION		
INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20014		
TOTAL MANYEARS: <div style="text-align: center;">1.9</div>	PROFESSIONAL: <div style="text-align: center;">1.5</div>	OTHER: <div style="text-align: center;">0.4</div>
SUMMARY OF WORK (200 words or less - underline keywords) <p> This project is designed to provide information on the mechanisms operating within <u>reflex</u> systems which include <u>alpha motoneurons</u> as the output link, as well as on the interconnection and interaction of different reflex systems. Particular consideration is given to correlations between <u>synaptic organization</u>, intrinsic neuronal properties, dynamic behavior and the <u>physiological</u> characteristics of the <u>muscle fibers</u> innervated by the motoneurons studies. </p>		

Project Description:

Objectives: This project is designed to provide information on the organization of those neuronal systems in the spinal cord of mammals which ultimately control the discharge of alpha motoneurons, and on the interaction of primary afferent and supraspinal descending systems in the control of information flow in the segmental motor mechanisms.

Methods Employed: The experimental work of this project primarily involves acute preparations (adult cats) studied either under anesthesia (mainly pentobarbital) or unanesthetized following destruction of the supratentorial brain (decerebrate preparations). In some experiments, intracellular recording techniques are used to record synaptic events produced in medial gastrocnemius motoneurons by activity in a variety of synaptic input systems, including primary afferent fibers from muscle and cutaneous receptors as well as in fibers descending from various supraspinal structures. Input pathways are activated either by electrical or natural stimuli. Other experiments have monitored motoneuron discharge patterns either by recording single motor unit electromyographic potentials (EMG) in target muscles or by recording motoneuron axon discharges in small filaments of muscle nerves.

A second series of experiments was completed during this fiscal year which involved deposition of the tracer material horseradish peroxidase in the motor nucleus of the medial gastrocnemius muscle through a precisely placed micropipette. After 24 hours' survival, cats so injected were sacrificed under anesthesia, perfused with fixative and the spinal cord processed for histological demonstration of the peroxidase tracer in spinal interneurons.

Major Findings:

- A. Organization of synaptic input to motor units of defined type.

During FY 1975, most of our attention in this phase of the project has been on the effect of cutaneous afferent input from the hind foot on recruitment patterns of medial gastrocnemius (MG) motor units. We have shown that, in unanesthetized decerebrate cats, motor units in the soleus muscle (a close synergist of MG composed almost exclusively of slow twitch [type S] motor units) are powerfully inhibited by sural nerve (cutaneous) afferents of low to medium electrical threshold, while many motor units in the MG are simultaneously excited in an equally powerful manner. Our major question, given this observation, was whether type S motor units within the MG motor unit pool might be inhibited by the sural stimulus while other, presumably fast twitch

(type F) motor units were excited. Our attempts to demonstrate such a reversal of unit recruitment pattern has involved production of enhanced activity in both MG and soleus motor unit pools by longitudinal vibration of either or both muscles (producing the tonic vibration reflex), and then conditioning this input by a short tetanic stimulation of the sural nerve. The discharge patterns of MG motor units have been studied using discrete electromyographic (EMG) recordings or by recording the discharge of small numbers of MG motor axons in a cut filament of the MG muscle nerve isolated just proximal to where the whole nerve enters the MG muscle.

Both recording techniques clearly demonstrate that many MG motoneurons are powerfully excited by the same sural nerve input that inhibits firing in synergist soleus motoneurons. We also have a number of EMG and nerve filament recordings which clearly suggest that some MG motoneurons recruited during the tonic vibration reflex are then inhibited by the sural input. However, it has been technically very difficult to provide unambiguous demonstration of the drop out of some MG neurons because of the massive additional firing produced in previously silent motoneurons by the sural stimulation. Nevertheless, even though the relatively clear recordings are few in number, they appear to be sufficient to establish the point that large reversals in recruitment pattern can in fact occur under the conditions of this experiment. This result is consistent with our hypothesis that the major factor controlling motoneuron recruitment is the organization of synaptic input to the motoneuron pool, rather than some factor(s) intrinsic to the motoneurons themselves. It is technically impossible at this time to identify the units inhibited by sural stimulation as type S, but there is indirect evidence that this is probably the case.

B. Anatomical identification of last-order interneurons.

This phase of the project was begun in FY 1975 and completed during FY 1976. A total of 15 cats were used, in which horseradish peroxidase type VI (HRP) was injected through a micropipette into the center of the MG motor nucleus in the spinal cord. After 24 hours, the animals were perfused with fixative under anesthesia and the spinal cord was processed for histological demonstration of the enzyme tracer. The sizes and positions of interneurons showing the tracer label were plotted on diagrams of the section outlines. This work has provided us with spinal cord maps indicating the positions and relative densities of interneurons, some of which must project to the medial gastrocnemius nucleus (and therefore presumably to MG motoneurons directly). We intend to use this information in the next phase of this project, which is to study individual interneurons that project directly to MG motoneurons of

defined motor unit type.

Significance to Biomedical Research and the Program of the Institute:

. With few exceptions, active movement of mammals in space is accomplished by motor units with motoneurons located in the spinal cord. Analysis of the central nervous system control of movement requires detailed understanding of the organization and interaction of input systems to the spinal cord segments, both from peripheral afferent sources and from supraspinal structures. There is now considerable evidence for the existence of functional specializations among the muscle fibers of different motor unit types, indicating rather precise patterns of motor unit "usage" during movements of various sorts. The long-range goal of the present project is to analyze the patterns of neuronal organization present in the spinal cord as they relate to motor unit type in order to further our understanding of how motor units, and therefore movements, are controlled. Such studies are of clear relevance to analyses of both normal and abnormal movement patterns in man and bear importantly on the interpretation of results of clinical neurophysiological investigations in normal human subjects, and in patients with neurological diseases.

Proposed course of the project:

Examination of the effects of cutaneous input on motoneuron discharge patterns during vibration reflexes will be concluded during this fiscal year. Major attention will then turn again to intracellular recording techniques with an investigation of the interaction between cutaneous input and descending input from various brain stem structures, as this interaction is manifest in MG motoneurons belonging to motor units of defined type. This work was begun several years ago but has progressed slowly, awaiting the acquisition of a signal averaging device which is now available. In addition, we intend to make use of the anatomical localization of presumed last-order interneurons in the spinal cord to search for individual interneurons that are connected monosynaptically with MG motoneurons. This work will also require the signal averaging system in order to study synaptic potentials produced by the recorded interneurons using the method of spike triggered averaging.

Keyword Descriptors:

Motoneurons
Motor units
Interneurons
Spinal cord

Publications:

Burke, R.E., Rymer, W.Z. and Walsh, J.V. Relative strength of synaptic input from short latency pathways to motor units of defined type in cat medial gastrocnemius. J. Neurophysiol. In press. 1976.

Burke, R.E., The physiology of alpha motoneurons and their synaptic input in relation to the problem of ALS. In: J. M. Andrews, R.T. John and M.A.B. Brazier, (eds.). Amyotrophic Lateral Sclerosis: Recent Research Trends. New York: Academic Press. In press. 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01687 08 LNLC
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) TECHNIQUES FOR MAKING CONNECTIONS WITH THE NERVOUS SYSTEM		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT Principal Investigator: Martin Bak, Electronics Engineer, LNLC, NINCDS Other Investigators: Robert E. Burke, M.D., Acting Chief, LNLC, NINCDS Clifford Christian, M.D., Behavioral Biology Branch, NICHHD Stephen Cohen, M.D., Behavioral Biology Branch, NICHHD George Dold, Electrical Engineer, LNLC Seth Goldstein, Ph.D. DRS, BEIB F. Terry Hambrecht, M.D., Fundamental Neurosciences Program, NINCDS J. A. Hoffer, Ph.D., Johns Hopkins University, Baltimore, Md. Gerald E. Loeb, M.D. Research Associate, LNLC; William B. Marks, Ph.D., Research Physiologist, LNLC; N. Mutsuga, M.D., Clinical Neurosciences Branch, NINCDS; George Norris, DRS, BEIB; Michael Salcman, M.D., Columbia Presbyterian Medical Center, New York, NY; Edward Schmidt, Ph.D. and Bruce Walmsley, Ph.D., LNLC, NINCDS		
COOPERATING UNITS (if any) Behavioral Biology Branch, NICHHD BEIB, Div. of Research Services, NIH; FNP, NINCDS; Johns Hopkins University, CNB, NINCDS; and Columbia Presbyterian Medical Center		
LAB/BRANCH Laboratory of Neural Control		
SECTION		
INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Md. 20014		
TOTAL MANYEARS: 3.1	PROFESSIONAL: 2.0	OTHER: 1.1
SUMMARY OF WORK (200 words or less - underline keywords) This project is intended to develop techniques for the acquisition and processing of neuroelectric signals from the central and peripheral nervous system in <u>acute and chronic neurophysiological</u> <u>preparations.</u>		

Project Description:

Objectives: Successful monitoring of neural activity from the peripheral or central nervous system requires the development of techniques as they apply to unique recording situations. These include both acute and chronic preparations in which stable and discriminable single unit activity is required, including animal preparations that are awake, comfortable and either free to move or moving with minimal restraint.

Methods Employed:

A. Materials suited for biological implantation.

The evaluation of the physical properties and biocompatibility of Parylene-C, iridium and certain medical grade silastic rubbers has continued.

B. Designs for a chronic recording intracortical microelectrodes.

1. An array of 4 "map pin" electrodes has been designed and implanted in the spinal cord of cats in the L6 region.
2. Histological evaluation of neural tissue after electrode implantation for varying periods of time up to one year is being performed in cat and monkey.
3. "Hair brush" electrode arrays containing three or more electrodes have been built and implanted for study of small neuronal pools and electrode to cell migration.
4. Glow discharge techniques have been applied to the surface of parylene to change its surface characteristics from hydrophobic to hydrophylic.

C. Microelectrode introducer capable of tracking the moving brain.

The introducer, a device developed previously and described in last year's Annual Report, has been used to record intracellularly from neurons in the cerebral cortex of monkey using a modified glass pipette electrode. Continued use of the device in humans is anticipated for studying epileptogenic activity of single neurons before removal of the damaged area.

D. Electrode arrays for chronic recording from nerve fibers.

Two rabbits have been implanted using the cuff electrode system in which the nerve to the tenuissimus muscle was captured and the 1 mm lead cable was brought directly through the skin, with favorable results as regards infection and long term recording performance of the implanted electrode system.

E. Chronic single unit recording from dorsal root ganglion.

The original electrode system has been modified with favorable results. The present system consists of 4-6 50 micron diameter 90% platinum-10% iridium alloy wires with Pyre-ML (polyimide) insulation. The wires are threaded through a thin-wall silastic tube leading from a connector saddle on the cat's back to a strain relief anchoring to the L7 dorsal spine. The wires are trimmed obliquely and inserted by hand into the L7 ganglion via a small laminotomy at the base of the L7 spine, leaving a loop of wire to take up relative motion during walking.

F. 16-Channel EEG telemetry system.

A continuous loop tape system was developed which allows selective recording on tape of a multiphase telemetry signal with a memory for EEG activity up to 4 minutes prior to the start of a clinical seizure. This data reduction system allows the clinician to concentrate on EEG activity just before and during a seizure without the burden of storing and examining large amounts of interictal EEG records.

G. Development of specialized substrates for nerve and muscle tissue culture.

Glass coverslips are coated with 700 angstrom layers of Parylene-C. The surface which is normally hydrophobic can be modified to any degree of hydrophilicity, producing any desired degree of cellular adhesion and propagation. Using photolithographic techniques, the surface can be arranged in patterns to cause, for example, alignment of randomly plated single myoblasts into parallel bands of muscle fibers.

H. Microelectrode drive system for extracellular recordings.

A stepping microdrive system has been developed that mounts on an animal's head for (single unit) extracellular recordings using metal microelectrodes. The electrode is moved in 5 micron steps over a range of 24 millimeters. The electrode position is displayed on a 4 digit L.E.D. readout in which the counter to the LED is reversible.

- I. Muscle puller employing servomechanism for constant tension under varying loads.

A motor driven muscle puller has been developed which utilizes a strain gauge to set and maintain steady tension on the muscle. Gating circuits have been built into the unit for automatic disabling while the same device measures muscle contraction during stimulation.

- J. 10-Channel high impedance amplifier system.

A custom designed 4 channel, low noise preamplifier with high input impedance has been developed and used which mounts directly on the back of freely moving cats for recording from microelectrodes in the spinal cord. Hybridization of the design to incorporate 10 amplifier stages is anticipated in order to miniaturize the system to a more functional size.

- K. Implantable strain gauge for monitoring muscle tension from associated tendon.

A miniature semiconductor strain gauge which is glued to a small uniquely designed stainless steel substrate has been implanted in cat. The intact tendon is fitted onto the device which then measures absolute forces generated by the muscle during normal animal movements.

- L. Analog delay for on-line confirmation of neuroelectric signals.

An analog delay circuit employing a recently developed charge-coupled shift register device has been developed and successfully used to obtain on-line visual confirmation of single units from records containing activity from several different neurons.

Major Findings:

Parylene-C has been found to be extremely non-reactive in a study in which eight cats were implanted with "map pin" electrodes insulated with glass or Parylene. The tissue reaction to Parylene after one year in situ (cerebral cortex) compared favorably to the reaction to glass-coated electrodes after only one month in place.

Modified "map pin" electrodes have recorded single unit activity in the spinal cord of cat for up to three weeks.

The microelectrode introducer has successfully recorded intracellular activity from the same cell in the cerebral cortex of monkey for up to 15 minutes.

Cuff electrode assemblies have recorded distinguishable afferent and efferent activity among approximately 100 nerve fibers for several weeks during normal movements in rabbit. Afferent and efferent fibers each fire at the beginning and end of flexion.

Chronically implanted electrodes in dorsal root ganglion of cat have recorded separable unit activity from 1 to 3 cells of several types for periods long enough to record the activity in normal locomotion and then acutely identifying the units.

The 16 channel EEG telemetry system has been used successfully in epileptic patients. In one patient suspected of having a unilateral temporal focus, a 10 hour telemetry record demonstrated bilateral temporal foci.

Hydrophilic Parylene-C without collagen or other coating appears to be an excellent substrate for most lines of neuronal and muscle tissue tested in tissue culture. Muscle fibers attached to the surface appear to have greater adhesion and can be maintained much further toward maturity than previously possible.

Muscle tension can be quantified during normal locomotion in freely moving cats by the use of a strain gauge assembly attached directly to the tendon.

Significance to Biomedical Research and the Program of the Institute:

The successful development of techniques for recording signals from the nervous system is essential to the success of ongoing experiments in the laboratory. These newly developed techniques are also beneficial to other laboratories involved in neurophysiological research and may have an impact in the development of prosthetic devices for the neurologically handicapped.

Proposed Course of Project:

Continued modification of the "map pin" electrode for application in other structures of the central nervous system such as the cerebellar cortex is anticipated. Also, studies designed to optimize the electrodes' performance for periods exceeding 6 months will be conducted with regard to histotoxicity of electrode materials and electrode to cell migration. Electrode arrays for peripheral nerve and dorsal root ganglion will be used to study neural activity with regard to locomotion in awake animals. The telemetry system will continue to provide EEG recordings from epileptic patients. Various patterns and surface properties of Parylene coated substrates for tissue culture will be explored in order to control the propagation and relative distribution of several

different cell lines. Plans to attach the strain gauge assembly to all three tendons associated with the tricep surae muscles in cats is anticipated to determine the correlation of integrated EMG to muscle tension. Some of the projects discussed need further development and work will continue on them. Further cooperation with the Neural Prosthesis Program is anticipated.

Keyword Descriptors:

Chronic single unit recording
Microelectrode
Hybridization
Glow discharge
Semiconductors strain gauge
EEG telemetry
Materials testing

Publications:

- Norris, G.F. and Schmidt, E.M.: An improved microelectrode drive system for neuroelectric monitoring. 28th ACEMB, 1975, p. 47.
- Loeb, G.E., Bak, M.J., Salcman, M. and Schmidt, E.M.: Evaluation of a new biocompatible dielectric coating: Parylene insulated chronic microelectrodes. 28th ACEMB, 1975.
- Bak, M.J., Loeb, G.E., Schmidt, E.M. and Salcman, M.: Parylene-C as a chronically stable, reliable dielectric coating for implanted electrodes. Society for Neuroscience, 1975, p. 1124.
- Goldstein, S.R., Bak, M.J., Oakley, J.C., Schmidt, E.M. and VanBuren, J.M.: An instrument for stable single cell recording from pulsating human cerebral cortex. Electroenceph. Clin. Neurophysiol. 39: 667-670, 1975.
- Bak, M.J. and Schmidt, E.M.: An analog delay circuit for on-line visual confirmation of discriminated neuroelectric signals. IEEE Trans. Biomed. Engng. In press.
- Hoffer, J.A. and Marks, W.B.: Long-term nerve fiber recording and stimulation in mammals. Neuroscience Abstracts, 1:244, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 701 NS 01688 08 LNLC
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) CORTICAL MECHANISMS OF VOLUNTARY MOTOR CONTROL		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT Principal Investigator: Edward M. Schmidt, Ph.D., Biological Engineer, LNLC Other Investigators: Martin Bak, Electronics Engineer, LNLC George Dold, Engineering Technician, LNLC Robert E. Burke, M.D., Acting Chief, LNLC Joan McIntosh, Physiologist, LNLC J. Stevenson Thomas, Ph.D., Staff Fellow, LNLC N. Mutsuga, M.D., Visiting Scientist, CNB, NINCDS		
COOPERATING UNITS (if any) Clinical Neuroscience Branch, NINCDS		
LAB/BRANCH Laboratory of Neural Control		
SECTION		
INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders and Stroke, Bldg. 36, Rm. 5A29, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 3.1	PROFESSIONAL: 1.4	OTHER: 1.7
SUMMARY OF WORK (200 words or less - underline keywords) This project is designed to investigate the behavior of individual neurons in the primate motor cortex during the production of spontaneous or conditioned limb movements in awake, minimally restrained animals. Long term <u>chronic recordings</u> from <u>single motor cortex neurons</u> are obtained with special microelectrodes. The <u>location and coding properties</u> of cortical neurons as they relate to various types of limb movement are being investigated.		

Project Description:

Objectives: The major goal of this project is to study the behavior of neurons in the motor cortex of primates in relation to spontaneous or conditioned limb movements. This involves the study of relationships between the firing patterns of cortical neurons and the measurable parameters associated with simple movements. One aim is to determine whether it is possible to extract enough information from the spike trains of small sets of cortical neurons to be able to predict the time course and amplitude of various motor responses. This requires a careful study of the types of cells (judged by response characteristics) to be found in the motor cortex, their frequency of occurrence, location and specific function.

Methods Employed: Monkeys were initially trained to move a handle that positioned a cursor light to one of eight spots. The monkey was required to match the position of his cursor light to one of eight target lights. When a match occurred for a prescribed length of time, a juice reward was delivered to the monkey. After implantation of up to a dozen "map pin" chronic microelectrodes in the motor cortex, the animal's cursor light was controlled by the firing pattern of a single cortical neuron. The task was the same as before except the handle was removed and the monkey was then allowed to make free arm movements. Correlations were sought between cortical neuron behavior and the type of movement selected by the animal.

Major Findings:

1) Monkeys are capable of controlling the firing rate of cortical neurons through operant conditioning techniques. Neurons that can be so conditioned are usually associated with discrete limb movements. Thus, we are probably conditioning limb movement even though the reward criterion is placed on the firing of a single neuron. Dividing the maximum firing rate of a cell up into eight discrete ranges, monkeys have been able to control the cell firing rate at an accuracy level as high as 94% when 80 random target positions were specified. If cortical neuron firing frequency were used to control an external device, the maximum information transfer rate so far achieved by a monkey has been 1.9 bits/sec. Doing the same task, but utilizing a wrist flexion or extension to move a handle results in an information transfer rate of 4.9 bits/sec. Thus, the maximum information transfer rate so far obtained for a cortical neuron was approximately 1/3 of the rate achieved with a trained movement.

2) Chronic "map pin" microelectrodes have also been implanted in the vicinity of an area in precentral motor cortex that had previously been rendered epileptogenic with alumina cream. With these electrodes it has been possible to record extracellularly from the same cortical neuron prior to, during, and after a clinical seizure. Before and during each

seizure "epileptic" neurons fired in bursts with short interspike intervals that have not been observed in "normal" cortical neurons. Only for a short period after each seizure did the neurons fire in a "normal" manner. The ability to monitor "epileptic" neurons for long periods should provide a method for evaluating the effects of drugs on the firing patterns of abnormal neurons and extending the period of "normal" firing.

3) A floating microdrive for extracellular recording has been developed and successfully tested in monkeys and in a human patient undergoing surgery for epilepsy. Microelectrodes are supported by novel frictionless air bearings that allow the electrode to follow cortical pulsations up to 3 mm. Recordings from single cells were obtained for up to 17.5 minutes before experimental protocol required moving the electrode to a new position.

Due to the stability of extracellular recordings obtained with the floating microdrive, the instrument was modified to utilize glass pipettes for intracellular recording. The instrument was successfully tested on monkeys where 23 intracellular recordings were obtained that lasted on the average of 7.4 minutes while the cortex was free to pulsate.

Significance to Biomedical Research and the Program of the Institute:

Through our studies we are obtaining a better understanding of the function of the motor cortex in relation to the generation and control of voluntary movement. The "map pin" electrodes have provided single unit recordings for many months which make feasible studies on movement under a variety of conditions. This work also has significance for the development of cortically-controlled prosthetic devices, although the electrodes are not yet satisfactory for obtaining prosthetic control signals where recordings for a number of years are required. The development of the floating microdrive has provided an instrument that can be used to search for "abnormal" neuronal activity in patients undergoing surgery for focal epilepsy. The instrument holds promise for more precisely localizing abnormal cortex.

Proposed Course of Project: Work will continue in the area of obtaining long-term chronic recordings from cells of the motor cortex. Different insulation materials will be investigated to determine if recording time can be extended. The main emphasis of the project will be on obtaining a better understanding of the function of the motor cortex. Conditioning of single cell activity will be combined with videotape monitoring of associated limb movements. By being able to characterize the activity of a cell under several different conditions

we should be able to better characterize the coding properties of movement-related motor cortex neurons.

Keyword Descriptors:

Chronic recording
Epilepsy
Floating microdrive
Human single cell recording
Intracellular recording
Motor cortex
Operant conditioning

Publications:

Goldstein, S.R., Bak, M.J., Oakley, J.C., Schmidt, E.M., and Van Buren, J.M.: An instrument for stable single cell recording from pulsating human cerebral cortex. Electroenceph. Clin. Neurophysiol. 39: 667-670, 1975.

Goldstein, S.R., Schmidt, E.M., Bierley, F.L. and Bak, M.J.: A gas bearing mechanism for stable electrical recording from individual neurons in pulsating human cerebral cortex. Trans. ASME J. Dynamic Systems, Measurement and Control 97: Series G, No. 3, 234-242, 1975.

Schmidt, E.M., Bak, M.J., McIntosh, J.S. and Thomas, J.S.: Control of operantly conditioned firing patterns of single precentral cortical cells. Soc. Neuroscience, 5th Ann. Mtg. 1975, p. 177.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02015 04 LNLG	
PERIOD COVERED July 1, 1975 through June 30, 1976					
TITLE OF PROJECT (80 characters or less) NEURAL PROSTHESIS PROGRAM					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT Project Officers: Frederick T. Hambrecht, M.D., Medical Officer, LNLG, NINCDS Karl Frank, Ph.D., Chief, Laboratory of Neural Control NINCDS Other Investigator: Herbert C. Lansdell, Ph.D. Clinical Research Psychologist, LNLG, NINCDS					
COOPERATING UNITS (if any) C & FR, NINCDS					
LAB/BRANCH Laboratory of Neural Control SECTION					
INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health Bethesda, Maryland 20014					
TOTAL MANYEARS:		PROFESSIONAL:		OTHER:	
SUMMARY OF WORK (200 words or less - underline keywords) This project was transferred to the Fundamental Neurosciences Program, NINCDS, in April, 1975 (See Contract Narratives N01-NS-0-2275; N01-NS-0-2276; N01-NS-0-2279; N01-NS-1-2286; N01-NS-3-2307; N01-NS-3-2313; N01-NS-2-2314; N01-NS-4-2331; N01-NS-4-2332).					

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02078 03 LNLc
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) INPUT-OUTPUT PATHWAYS OF DORSAL ROOT GANGLION CELLS		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT Principal Investigator: Gerald E. Loeb, M.D., Medical Officer, LNLc, NINCDS Other Investigators: None		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neural Control SECTION		
INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders & Stroke, Bldg. 36 Rm. 5A29 Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1
SUMMARY OF WORK (200 words or less - underline keywords) The presence of a small percentage of myelinated <u>afferents in</u> <u>ventral roots</u> has been demonstrated. The techniques developed <u>have been adapted for recording from afferents during normal</u> <u>movements.</u>		

Project Description:

The results presented in the previous report for FY 1975 were prepared for publication and for presentation at the annual Society for Neuroscience Meeting. This project has been terminated. The further work on chronic recording methods is described in another project report this year (Neuron Activity During Locomotion; Project No. Z01 NS 02080 02 LNLc).

Publications:

Loeb, G.E., "Decreased conduction velocity in the proximal projections of myelinated dorsal root ganglion cells in the cat", Brain Research, 103:381-385, 1976.

Loeb, G.E., "Ventral root projections of myelinated dorsal root ganglion cells in the cat", Brain Research, 106:159-165, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02079 03 LNLC
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) MODELS OF NEURAL INTERACTIONS		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT Principal Investigator: William B. Marks, Ph.D., Research Physiologist, LNLCC Other Investigators: None		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neural Control		
SECTION		
INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders and Stroke, NIH, Bldg. 36, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.4	OTHER: 0.1
SUMMARY OF WORK (200 words or less - underline keywords) Hidden source variables are being estimated in model systems by forming <u>independent functions of the data variables</u> . The <u>response of</u> the membrane potential of <u>neurons to extracellular pulsed fields</u> is being <u>calculated</u> .		

Project Description:

Objectives:

1. To develop the hypothesis of independent sources. This hypothesis states that correlated data variables can sometimes be considered as functions of hypothetical independent source variables and that these source variables may be recovered as independent functions of the data variables.
2. To calculate the responses of neurons to externally applied fields.

Methods Employed: Mathematics and computer modeling, as described in earlier project reports concerning projects I and II.

Major Findings:

Six results have been mentioned in the previous two project reports which utilize independent sources. Each result is concerned with calculating a matrix L which will recover linearly mixed source variables from data. These results were briefly:

1. If the source variables are non-Gaussian, a matrix which generates from the data new independent variables will equal L .
2. Two formulas giving L directly from the 2nd and 3rd cross moments of the data.
3. Formulas for the number of data samples required to calculate L using No. 2 above.
4. Simple conditions on the new variables which L must satisfy.
5. Two "growth rules" which guide an initial guess for the value of L to a final correct value.

In writing up No. 2 above for publication, subtle errors have been detected and corrected. The formulae have been carefully tested and used successfully when the number of source and data variables had five different values, up to 5 and 7 respectively. They are now written in publishable final form.

One of the growth rules in No. 5 has been reprogrammed for a small computer so that larger problems can be computed inexpensively. The first such problem attempted revealed that features for retinal patterns cannot be calculated until the method is modified to apply

when the occurrence of a feature is not entirely under the control of the source variables, but is subject to some "noise".

Significance to Biomedical Research and the Program of the Institute:

1. Some neurons may use the principle of independent sources to adjust the strength of their interconnections.
2. The response of neuronal membranes to pulsed fields from an electrode on the surface of the brain must be known before we can assess the efficiency of the electrodes presently being used as auxiliary sensory channels.

Proposed course of project:

- a. Results No. 1,2,3,4 and 5 can now be published in four papers. It has been very difficult to finalize No. 2, and now that this is done, Nos. 1 & 2 can be published. Nos. 3,4 and 5 will be much less difficult to complete and publish.
- b. An effort will be made to generalize the results in objective 1 to accept data that are not completely determined by the source variables, but which have some intrinsic variability. This would greatly enhance the usefulness of these methods.
- c. The technique used in objective 2 has been described in an earlier report. Now it will be used to calculate space and time profiles of membrane potential responses to a variety of applied pulses for neurons in a variety of positions with respect to the stimulating electrode, and the results published. This will also be presented in a symposium on sensory prostheses.

Keyword Descriptors:

Factor analyses
Feature detection
Extracellular stimulus

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE	U.S. DEPARTMENT OF	PROJECT NUMBER
PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE	
	PUBLIC HEALTH SERVICE	
	NOTICE OF	
	INTRAMURAL RESEARCH PROJECT	Z01 NS 02080 03 LNLC
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) NEURON ACTIVITY DURING LOCOMOTION		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT Principal Investigator: William B. Marks, Ph.D., Research Physiologist, LNLC Principal Co-Investigator: Gerald E. Loeb, M.D., Research Associate, LNLC Other Investigators: Bruce Walmsley, Ph.D. Visiting Fellow, LNLC Martin Bak, Electrical Engineer, LNLC		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neural Control		
SECTION		
INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders and Stroke, NIH, Bldg. 36, Bethesda, Maryland 20014		
TOTAL MANYEARS: 1.4	PROFESSIONAL: 1.0	OTHER: 0.4
SUMMARY OF WORK (200 words or less - underline keywords) The activity of spinal neurons is being monitored during <u>normal locomotion</u> , using <u>multichannel chronic nerve fiber electrodes</u> for spinal roots, and <u>chronic wire electrodes</u> for cell bodies, in order to better understand the role of these neurons in natural motor control.		

Project Description:

Objectives: The major goal of this project is to examine directly the roles of spinal neurons and primary afferent fibers in normal movement which up till now have been inferred from paralyzed or deenerate acute preparations. The principle current emphasis is on incoming activity in dorsal roots, and the main questions relate to the role of afferents as part of a system of servo-mechanisms; spinal reflexes elicited by both cutaneous and proprioceptive afferent activity are now known to have a profound effect on locomotory patterns.

Methods Employed:

1. Calculations of potential distributions of myelinated fibers were made using the Basic language on the DCRT DEC-10 computer.
2. Tube electrodes used in these experiments were described in last year's project report. The back pack and recording system are described below.
3. For recording from the dorsal root ganglion (DRG), insulated fine wires were inserted into the L7 DRG of the cat via a small laminotomy. These electrodes, together with a number of EMG leads implanted in leg muscles, are terminated in a connector affixed to the cat's back.
4. The recording system now consists of a multi-channel amplifier in a back-pack, a 14-channel FM tape recorder, a treadmill, a video tape recording system, a PDP-12 computer, a timing synchronizer, and a flexible interconnect arrangement. Video records are used to locate significant epochs in the movement to 1/60 sec. accuracy, for comparison with unit activity using the computer.

Major Findings:

1. We now have published predictions for the node currents of mammalian nerve fibers of various diameters, and the associated extracellular potential fields within nerve bundles, which can both guide efforts to record from these fibers and suggest basic experiments on them. The prediction of large longitudinal fields near nodes suggests a longitudinal electrode configuration as optimal for chronic recording.
2. Insulated fine wires simply cut off and inserted into a dorsal root ganglion can record separable unit activity from 1-3 DRG

cells of several types, stable at least long enough for recording activity in normal locomotion and then acutely identifying the units.

3. By capturing the nerve to the tenuissimus muscle, (a knee flexor and hip extensor) in the rabbit in two of our tube electrodes in series, we have recorded distinguishable afferent and efferent activity among about 100 nerve fibers for weeks during normal movements. Afferents and efferents were each found to fire at the beginning and end of knee flexion.

Significance to Biomedical Research and the Program of the Institute:

The study of mammalian locomotion has been concentrated on the cat hind limb, where considerable knowledge is already available concerning the physiological and anatomical properties of the muscles, motor neurons, afferents, and spinal reflexes. However, the details of the functioning of this system during normal locomotion under cerebral control can at present only be inferred, giving rise to a number of competing control theory hypotheses. This new methodology should provide data needed for testing such hypotheses and formulating new ones. An understanding of the normal control of movement is essential to understanding a number of degenerative diseases of the spinal cord (e.g. ALS, transverse myelitis, etc.) affecting locomotion. A longer range application of the technique of chronic afferent monitoring is in the field of functional neuromuscular stimulation (FNS). Sophisticated devices designed to restore motor function by bypassing CNS lesions (e.g. paraplegics) will probably require some form of closed loop servo-control utilizing transducers of muscle length and tension and of skin pressure. If it proves possible to stably record afferent activity over long periods of time, such biological transducers could improve the function and simplify the design and implantation of complete FNS systems.

Proposed course of project: For the wire electrodes, a series of semi-chronic experiments is planned in which implantation in the DRG and recovery is followed by a recording session during which locomotion and consequent afferent activity is assessed as previously described. Immediately afterward, while still connected to the amplifier system, the animal will be anesthetized and the muscles and nerves of the hind limb dissected out to allow characterization of the units recorded, including receptor modality, site of endings, conduction velocity, and sensitivity. The next phase of the experiments is projected to include the study of afferent activity during perturbations

of the gait, looking particularly at the question of phase dependent reflexes. A longer range goal includes the simultaneous recording of motor unit activity from similarly implanted chronic microelectrodes in the spinal cord (apparently feasible but considerably less stable according to one preliminary experiment to date).

Chronic recording experiments on nerve fibers will continue and improvements to resolve units will be sought as follows:

1. Standard and smaller tube electrodes will be used to capture dorsal rootlets, and the rootlets will be partly crushed to reduce the number of active fibers.

2. Fine wires will be inserted into the captured rootlets longitudinally, either to detect the predicted longitudinally extended unit potentials, or as additional contacts recording mixtures of units different from those seen by the standard side-tube contact. Signals from several such contacts might yield resolvable units when rightly combined.

3. Tube electrodes will be adapted to attempt to capture fibers within the spinal cord. Longitudinal contacts will also be inserted into the cord.

4. Successful chronic nerve fiber preparations may be stable for many days, and if so, will be used to examine the change in the activity of muscle afferent fibers over longer time periods.

Keyword Descriptors:

locomotion
chronic recording
spinal cord
somathesis
afferent neurons

Publications:

Marks, W.B. and Loeb, G.E. Action currents, internodal potentials, and extracellular records of myelinated mammalian nerve fibers derived from node potentials. *Biophys. J.* (1976), In press.

Hoffer, J.A. and Marks, W.B.: Flexor afferent and efferent activity during locomotion in intact rabbits. International Conference on Neural Control of Locomotion, 1975, p. 43.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02160 02 LNLG
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) INTRINSIC PROPERTIES OF MOTOR UNITS		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT Principal Investigator: Robert E. Burke, M.D., Acting Chief, LNLG Other Investigators: Richard F. Mayer, M.D., Guest Worker, Univ. of Maryland, Baltimore, Md.; V. Reggie Edgerton, Ph.D., Dept. of Kinesiology, Univ. of Calif., Los Angeles, CA.; Kenro Kanda, M.D., Visiting Fellow, LNLG; Bruce Walmsley, Ph.D., Visiting Fellow, LNLG; Peter Strick, Ph.D., Staff Fellow, LNP, NIMH; C. C. Kim, Ph.D., Visiting Fellow, LNP, NIMH		
COOPERATING UNITS (if any) Univ. of Maryland Sch. of Medicine, Dept. of Neurology, Baltimore, Md. Univ. of California, Dept. of Kinesiology, Los Angeles, Calif. Laboratory of Neurophysiology, NIMH, Bethesda, Md.		
LAB/BRANCH Laboratory of Neural Control		
SECTION		
INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders and Stroke, NIH, Bldg. 36, Bethesda, Maryland 20014		
TOTAL MANYEARS: 1.8	PROFESSIONAL: 1.5	OTHER: 0.3
SUMMARY OF WORK (200 words or less - underline keywords) This project is designed to provide information on the ranges and distributions of the electrophysiological and morphological characteristics of <u>alpha motoneurons</u> and of the interrelated mechanical, histochemical and morphological properties of the <u>muscle fibers</u> innervated by them (i.e., the muscle unit). The <u>motor unit</u> populations in normal animals are compared with those in animals after various conditioning treatments.		

Project Description

Objective: This project is designed to provide information on the ranges and distributions of electrophysiological, mechanical, histochemical and morphological characteristics of individual motor units in mammals, principally in the cat. In addition, we have investigated the changes in the properties produced by altered usage in adult animals.

Methods Employed: For the most part, acute experiments are carried out in anesthetized cats, using intracellular recording and stimulation of alpha motoneurons to ensure functional isolation of single motor units. The electrophysiological properties of the innervating motoneurons can be examined using conventional techniques and then the mechanical properties of the innervated muscle unit is assessed by recording tension production at the appropriate tendon during stimulation of the motoneuron through the intracellular pipette. For specific experimental series, various conditioning situations have been used to alter the activity of the motor unit pool under study, such as surgical removal of synergist muscles or immobilization of the appropriate limb by joint pinning.

A second series of experiments has been done during FY 1976 in which the tracer substance horseradish peroxidase type VI (HRP) was injected under sterile conditions into the medial gastrocnemius (MG) muscle, as well as into the soleus muscle of the opposite hindleg, in cats under pentobarbital anesthesia. After a four day survival period, the animals were perfused with fixative solution under deep anesthesia and the spinal cord was processed by frozen section techniques for demonstration of HRP staining in motoneurons.

Major Findings:

A. Motor units after immobilization atrophy.

A study of medial gastrocnemius (MG) motor units properties after long-term (6 - 7 months) partial immobilization of the left hind leg at knee and ankle was begun during FY 1975. During the past year, four cats surgically prepared with steel pins through the knee and ankle joints of the left leg, were examined with our usual techniques for motor unit analysis. The mechanical properties of unit muscle fibers were assessed using intracellular stimulating microelectrodes to ensure functional isolation of single units. The data from units in immobilized MG muscles were compared to a large series of MG units obtained from normal cats of about the same size and body weight. We found striking diminution in

the average tetanic and twitch tensions of type FR and of type S motor units, with little change in twitch contraction times or motor unit resistance to fatigue. The average tension output of type FF units was much less decreased. Motor units in the atrophic MG muscles (wet weight averaged about 60% of that of MG muscles on the unoperated side) were still identifiable as to motor unit type by the same criteria applied to units in normal animals.

The ankle extensor muscles MG, lateral gastrocnemius and soleus were all removed from both the operated and unoperated legs in each animal and frozen at -160°C for histochemical analysis. The cross section area of fibers in matched regions of MG on operated and unoperated sides were compared. The most striking degree of atrophy (measured in terms of fiber cross-section area) was found among the fibers which showed the histochemical profile characteristic of type FR muscle units. There was in general somewhat less fiber area decrease in fibers presumed to belong to type S units. The fibers with the histochemical profile of type FF units showed the least change in fiber area. Despite the striking atrophy in many FR and S unit fibers, the histochemical profiles and the overall mosaic of fiber types in the MG were essentially the same as found in normal muscle.

The electrophysiological properties of the alpha motoneurons of atrophic motor units appeared to be normal as far as they were tested. However, the maximum amplitude of group Ia monosynaptic excitatory postsynaptic potentials were less than expected (compared to normal data in the respective motor unit groups).

These findings indicate striking differential degrees of atrophy in the various groups of motor units normally present in the cat MG, with the greatest atrophy found among the fatigue resistant S and FR unit groups. The data also suggest that immobilization may produce some long-term decrease in the strength of synaptic action on motoneurons from muscle stretch receptors. These findings clearly required further study and during the past year, a second series of cats has been prepared in our Laboratory using somewhat larger steel pins than in the first series to give more complete immobilization particularly at the knee. Some of these animals will be examined at short survival periods (3 to 6 weeks) and the rest will be studied after 4 to 6 months of nearly complete limb immobilization.

B. The anatomical characteristics of alpha and gamma motoneurons making up the medial gastrocnemius and soleus motor nuclei in the spinal cord.

During the past year, we have studied the morphology of the MG and soleus motor nuclei using the exogenous protein tracer horseradish peroxidase (HRP), which labels neurons by retrograde transport. We injected relatively large doses of HRP (50 mg total for MG; 25 mg for soleus) throughout the respective muscles. Both alpha and gamma motoneurons (identified by measurement of cell soma diameters) are labeled. The gamma motoneurons are in general more heavily labeled with HRP than are alpha cells, and also exhibit larger labeled granules. This finding may provide a means of identifying alpha and gamma motoneurons in sections suitable for electron microscopy. Alpha and gamma motoneurons are intermingled through the MG and soleus motor nuclei. However, there is a clear rostral predominance of large alpha cells in the MG nucleus which matches our previous finding that the somatotopically-related dorsal margin of the MG muscle contains mainly fibers belonging to type FF motor units. The type FF units are clearly innervated by the larger alpha motoneurons on the basis of other physiological criteria. Previous evidence has suggested a rostral to caudal somotopy in the MG nucleus matching a dorsal to ventral margin location of innervated muscle units in the MG muscle.

Significance to Biomedical Research and the Program of the Institute:

Analysis of the control of movement by the central nervous system requires consideration of the properties and functional specialization of motor units, as these are the quantal elements from which all skeletal movements are composed. Study of the interrelation between the intrinsic properties of motor units, including both the motoneuron and muscle unit portions, and the organization of synaptic input to the same units has aided our understanding of the control problem and has suggested new avenues for research. In addition, elucidation of the detailed interrelation between the physiological, morphological and histochemical characteristics of muscle units in animal muscle has immediate relevance to investigations of human neuromuscular disease, in which electromyography and muscle histochemistry play important diagnostic and research roles. There is growing evidence that the basic pattern of motor unit organization in animals and man is similar in principle.

Proposed course of the project:

It is anticipated that the series of immobilized animals now being prepared should provide material to complete this phase of the investigation of immobilization atrophy. Analysis of physiological data and of the histochemical preparations will carry the project into the next fiscal year. The study of the anatomy of the MG and soleus motor nuclei is essentially complete with regard to

light microscopy. We have initiated a further collaborative study of the ultrastructural correlates of these results which, if successful, should open a further avenue for investigation. Several pilot experiments during the past year were designed to examine motor unit properties in animals subjected to partial spinal cord section and consequent long-term alterations in muscle tone. It is anticipated that such collaborative studies will continue.

Keyword Descriptors:

muscle fibers
contractile properties of muscle
motoneurons
"trophic" effects

Publications:

- Burke, R.E.: A comment on the existence of motor unit "types"
In: Tower, D.B. and Brady, R.O. (Eds.) The Nervous System
Vol. 1 The Basic Neurosciences. New York, Raven Press, 1975,
pp. 611-619.
- Burke, R.E.: The motor unit viewed from above. Bradley, W.G.,
Gardner-Medwin, D. and Walton, J.N. (Eds.), In: Recent Advances
in Myology (Proc. IIIrd International Congress on Muscle Diseases.)
Amsterdam: Excerpta Medica, 1975, pp. 64-69.
- Burke, R.E., Rudomin, P. and Zajac, F.E. The effect of activation
history on tension production by individual muscle units.
Brain Res. In press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02161 02 LNLc
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) CONTROL OF SINGLE MOTOR UNIT FIRING PATTERNS IN HUMANS		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT Principal Investigator: Edward M. Schmidt, Ph.D., Biological Engineer, LNLc Other Investigators: F. Terry Hambrecht, M.D., Medical Officer, EAP, FNP J. Stevenson Thomas, Ph.D., Staff Fellow, LNLc, NINCDS Joan S. McIntosh, Physiologist, LNLc, NINCDS		
COOPERATING UNITS (if any) Fundamental Neurosciences Program, NINCDS		
LAB/BRANCH Laboratory of Neural Control		
SECTION		
INSTITUTE AND LOCATION : National Institute of Neurological and Communicative Disorders and Stroke, Bldg. 36, Rm. 5A29, Bethesda, Maryland 20014		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.7	OTHER: 0.3
SUMMARY OF WORK (200 words or less - underline keywords) The objectives of this study have been to obtain a better understanding of the organization of human <u>motoneuron pools</u> , and to determine the extent of control a human has over the <u>recruitment order</u> and firing frequency of <u>single motor units</u> .		

Project Description:

Objectives: The objectives of this study are twofold: the first being to obtain a better understanding of the organization of motoneuron pools, and the second to determine the extent of control a human has over the firing frequency of a single motor unit.

Methods Employed: Bipolar platinum wire electrodes (1 mil insulated wire exposed only at the tips) are introduced into selected muscles of the hand and arm with a hypodermic needle. The electrodes normally record the activity of several motor units in the muscle. Potentials generated by different motor units usually have different waveshapes that allow one to separate the multi-unit data into single unit activity with "on-line" discriminators. The activity of a single motor unit is fed back to the subject in terms of an auditory tone and a visual display of waveshape. The subject is then asked to control the firing frequency of the motor unit and also try to modify the order of recruitment of motor units.

Major Findings:

- 1) Some subjects have been able to consciously modify the recruitment order of motor units that are normally recruited near each other. Units that are widely separated in normal recruitment threshold have not been recruited in reverse order by any subjects.
- 2) In some muscles the recruitment order of motor units appears to be a function of previous activity of the motor unit. After a unit has fired for some time it becomes difficult to recruit that unit and another unit becomes easier to recruit. This phenomena appears to be analogous to "rotation" among motor units, which is a point of some past controversy.

Significance to Biomedical Research and Program of the Institute:

The analysis of single motor unit activity in humans will provide us with a better understanding of the organization of the neuro-muscular system, and this work complements animal studies on motor units in this Laboratory. The ability to record these signals reliably may also provide a source of signals that could be used to control stimulation of paralyzed muscles or control a prosthetic

device in a more refined method than is possible with gross EMG electrodes.

Keyword Descriptors:

Single motor units
Recruitment order of motor units
Voluntary control of motor units

Proposed Course of Project:

This project will be terminated on June 30, 1976.

Publications: None

ANNUAL REPORT
July 1, 1975 through June 30, 1976
Laboratory of Neurophysiology, Intramural Research
National Institute of Neurological and Communicative Disorders
and Stroke

M. G. F. Fuortes, M.D., Chief

Six scientists have joined the LNP during the fiscal year; eight have left the Laboratory so that the staff includes now seventeen professionals. The supporting staff consists of one secretary and three technicians, as in previous years. Seven scientists presently at LNP do not occupy positions. As a consequence, of the sixteen positions assigned to the Laboratory, only fourteen have been occupied during most of the fiscal year. Candidates for these openings are now available and it is expected that the positions will be filled around July 1976.

Many activities have been reorganized taking advantage of the PDP-11 computer which was purchased last year. The computer is now connected to several electrophysiological setups, and is used on-line for A to D conversion, storing, plotting and analyzing data. Graphic programs have been developed in collaboration with the Section on Technical Development. These programs provide a rapid and economical method for preparing illustrations and slides of remarkably high quality. The larger computer at DCRT was extensively used for model building and other mathematical applications.

The experimental studies include work on membranes, synapses, and receptors. The problems under consideration are investigated by making use of anatomical, electrophysiological or spectrophotometric methods.

Membrane properties are studied applying voltage-clamp techniques to molluscan neurons. The conductances to monovalent and divalent cations are analyzed in neurons which display pacemaker or bursting activity. The effects of various drugs and biochemical compounds on such activities have been investigated and it is hoped that the results will lead to conclusions applicable to the interpretation of epileptic activities.

Spectrophotometric studies of visual pigments have been successfully continued. Comparative investigation of these pigments of fish have been performed in collaboration with the University of Montreal. The work has provided new interpretations of the absorption properties and evolutionary development of visual pigments.

Studies on the retina are still actively pursued with the aim of understanding the mode and actions of photoreceptors and to clarify the main principles of organizations of retinal cells. The collaboration established in past years with the University of Cambridge, England and with the Italian National Research Council (C.N.R.) is being continued. The anatomical studies performed this year have revealed new properties in the synaptic organization of bipolar and amacrine cells. Physiological experiments have been performed on photoreceptors and have been useful in clarifying the role of calcium and other ions in the transduction process. Other experiments have shown that rods and horizontal cells of Bufo Marinus often develop voltage oscillations which strongly suggest the existence of reverberation pathways between these few cell types. The work on receptors is now being supplemented by noise analysis.

The major results of the studies mentioned above have been reported in seventeen articles published during the year.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01659-08 LNP
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PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Synaptic Contacts of Visual Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. Lasansky Research Biologist LNP NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

Section on Cell Biology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

1.5

PROFESSIONAL:

1

OTHER:

0.5

SUMMARY OF WORK (200 words or less - underline keywords)

Bipolar cells in the salamander retina can be classified into two major types according as to whether they are hyperpolarized or depolarized by illumination of the center of their receptive field. In either case, illumination with an annulus of light results in a response of opposite polarity. This antagonistic effect of the surround, however, is seen only when the center is dark or dimly illuminated; when the center is brightly illuminated, flashing an annulus evokes no response. The circuitry underlying this interaction between center and surround is being studied at present.

Project Description:

Objectives: To investigate the fine structure and function of the synapses between receptors and other retinal neurons.

Methods Employed: Electron microscopy combined with silver impregnations by the method of Golgi. Electrical recordings with intracellular microelectrodes.

Major Findings: The responses to light of bipolar cells were intracellularly recorded in the retina of the larval tiger salamander. The identity of the impaled cells was established by means of Procion yellow injections. As already known for other retinae, two main types of bipolar cell responses could be distinguished, since in one type, illumination of the center of the receptive field resulted in hyperpolarization and in the other produced depolarization. In either instance illumination of the surround elicited a response of opposite sign to that evoked by illumination of the center. The surround response could be observed without any preceding or simultaneous illumination of the center. Because of the antagonistic effect of the surround, responses to large circles of dim light were of smaller amplitude than those evoked by small circles. With bright flashes, however, the responses to large light circles had the same amplitude as those to small circles. Such an observation was interpreted as a suppression of the surround antagonism when the center is brightly illuminated, and this could be shown to be the case when a light annulus was flashed during steady illumination of the center with a small light circle: as the intensity of the light circle was increased, the response to the annulus decreased and finally disappeared.

Significance to Bio-medical Research and the Program of the Institute: It is hoped that these observations will help in identifying the mechanisms of synaptic transmission between photoreceptor cells and second order neurons, and provide a better knowledge of the neuronal networks involved in the processing of visual information within the retina.

Proposed Course of the Project: The responses of bipolar and horizontal cells will be compared following stimulation with various light patterns, in order to understand the circuitry underlying the suppression of the surround effect on bipolar cells when the center is brightly illuminated.

Publications:

Lasansky, A: Interactions between horizontal cells of the salamander retina. Invest. Ophthal. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02153-02 LNP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Description of an Oscillatory State in the Vertebrate Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Normann	Staff Fellow	LNP NINCDS
OTHER:	J. Pochobradsky	Visiting Fellow	LNP NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

Section on Cell Biology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0

SUMMARY OF WORK (200 words or less - underline keywords)

An unconventional form of electrical activity was observed in the rod photoreceptors of the vertebrate retina. This activity manifested itself in the form of periodic variations in membrane potential. Various intracellular microelectrode experiments were undertaken in the retina of the rod, Bufo Marinus, to decide whether these oscillations resulted from single cell mechanisms or from interactions between the photoreceptors and the second order retinal neurons, the horizontal cells. It was observed that the receptive field of the rod oscillations was similar in size to the receptive field of the horizontal cell light response and much larger than the receptive field of the rod light response. The chemical, sodium aspartate, was delivered to the retina and the oscillations were eliminated. Sodium aspartate blocks synaptic transmission from rods to the horizontal cells. The results of these experiments support the interaction hypothesis, and as such, represent the first experimental verification of the existence of a neural feedback pathway from horizontal cells to a pure rod type photoreceptor.

Project Description:

Objectives: The principal objectives are 1) to describe the conditions which elicit the oscillatory state and 2) to determine whether the oscillatory state is a single cell or a network phenomenon.

Methods Employed: Intracellular recordings were performed in the eyecup of Bufo Marinus using high resistance microelectrodes. Retinal temperature was controlled with a Peltier device. The retina was kept in an oxygenated or anaerobic state by the use of externally applied gases. Retinal light stimulation was accomplished with a two channel photostimulator. The color, size, shape, and intensity of the light stimulation could be controlled independently in the two channels. The experiments were performed using two different preparative techniques; the eyecup preparation and the isolated, inverted and perfused retina. The latter preparation was used to test the effects of various chemicals on the electrical properties of the retinal neurons.

Major Findings: The retinal neurons of Bufo Marinus can exist in at least two states. In the conventional state, the responses recorded from the rod photoreceptors, the horizontal cells, the bipolar cells and the ganglion cells are similar to those described in other vertebrate retinæ. The other state is characterized by oscillatory responses. In this state, the retinal neurons of Bufo exhibit either transient oscillations following test flash stimulation or sustained oscillations which can persist for up to fifteen minutes. The oscillations were typically between 1 and 3 Hz. In the eyecup preparation, the rod oscillations were approximately sinusoidal while the horizontal cell oscillations usually exhibited rectification: oscillating components which were more depolarizing than the dark-adapted baseline were observed while hyperpolarizing components were absent. In the isolated retina instead both rod and horizontal cell oscillations were sinusoidal. The oscillatory state was transient and lasted typically for three to fifteen minutes. Before and after the oscillatory state was observed, the more conventional behavior occurs.

Experimental conditions which augment or inhibit the oscillatory state were described previously in the project description of last years project report. It was also found that perfusion of the retina with HEPES buffer always triggered transient oscillations in the rods and horizontal cells.

The experiments described below were undertaken to decide whether the rod oscillations were a result of single cell mechanisms or a result of interactions between the rods and the horizontal cells. In the first class of experiments, the recep-

tive field properties of rod oscillations were investigated. It was found that small diameter spots which produced maximal excitation of the underlying rods would neither provide much horizontal cell excitation nor elicit oscillations in the rods. Enlarging the spot diameter from 60 to 1200 microns produced large rod responses, large horizontal cell responses and often oscillations in both rods and horizontal cells.

The second class of experiments were designed to study the effect of a block of synaptic transmission from rods to horizontal cells with the agent, sodium aspartate. It was observed that 2 mM sodium aspartate eliminated the horizontal cell response but had little effect on the rod response. In these conditions oscillations were completely eliminated in both rods and horizontal cells. The oscillations could be restored upon returning to the normal ringers solution.

The results of these experiments support the hypothesis that the oscillations arise from interactions between horizontal cells and rod photoreceptors. A precedent for such a feedback pathway from horizontal cells to cone type photoreceptors in the turtle retina has been established by earlier work done in this laboratory.

Significance to Bio-medical Research and the Program of the Institute: This project has significance at two levels. First, at the basic level, the oscillatory state was used to obtain the information about neural interactions in the retina, i.e., the oscillations appear to result from excitatory and inhibitory interactions between the rods and horizontal cells. Second, at the applied level, the retinal oscillations which occur spontaneously and transiently might serve as a model system for testing drugs used as antiepileptics. Also, since the oscillations appear to result from neural interactions, a detailed understanding of the oscillations and the conditions which generate the oscillatory state might provide basic insight into the mechanisms of epilepsy.

Proposed Course of the Project. Project is completed.

Publications:

Normann, R.A. and Pochobradsky, J.: Oscillations in rod and horizontal cell membrane potential; evidence for feedback to rods in the vertebrate retina. J. Physiol. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02221-01 LNP
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Ionic Mechanisms of Phototransduction in Rods of the Vertebrate Retina		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <div style="display: flex; justify-content: space-between; padding: 10px;"> PI: R. A. Normann Staff Fellow LNP NINCDS </div>		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neurophysiology		
SECTION Section on Cell Biology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: <div style="text-align: center;">0.5</div>	PROFESSIONAL: <div style="text-align: center;">0.5</div>	OTHER: <div style="text-align: center;">0.0</div>
SUMMARY OF WORK (200 words or less - underline keywords) The primary action of <u>light stimulation</u> on the <u>electrical properties</u> of <u>rod type photoreceptors</u> will be investigated using <u>electrophysiological techniques</u> . The study will be performed in the retina of the toad, <u>Bufo Marinus</u> . A <u>retinal perfusion system</u> has been developed which allows rapid changing of the ionic composition of the fluid bathing the photoreceptors. The effects of <u>monovalent</u> and <u>divalent cations</u> on the kinetics of the <u>photoresponse</u> will be investigated.		

Project Description:

Objectives: The principal objective of this study is to characterize the ion species that are involved in the transduction of light stimulation into the electrical response of the photoreceptor.

Methods Employed: Intracellular recordings of electrical potential and conductance will be performed in the isolated retina of Bufo Marinus using glass capillary microelectrodes. The retina is mounted in a chamber which allows perfusion of the photoreceptor with solutions of various ionic compositions. Dissection of the preparation and microelectrode positioning is accomplished using infrared visualization provided with commercially available infrared to visible image converters.

Major Findings: The initial phase of this study was concerned with implementation of the experiments and determination of their feasibility. It was observed that photoreceptors can be impaled and recorded from while the perfusate was switched to a number of different ionic compositions. The total wash-out time of the perfusion chamber is about two minutes and satisfactory impalement of rod photoreceptors for periods as long as 45 minutes using these techniques could be maintained.

Preliminary results are as follows:

Effects of reducing external sodium. External sodium was reduced by replacement of sodium chloride with choline chloride or with tris. Decrease of external sodium causes rod hyperpolarization and reduction of response amplitude with little effect on response kinetics.

Effects of increasing external potassium. External potassium was increased with equimolar reduction of glucose (which was present in the "normal" Ringers solution). Increased potassium causes rod depolarization and reduction of the response amplitude. For brighter test flashes, the initial peak of the response experienced greater reduction than the plateau component of the response.

Effects of extrinsic currents: Hyperpolarizing and depolarizing extrinsic currents up to 3×10^{-10} A were passed through the microelectrode and their action on the rod response was studied. Hyperpolarizing currents had little effect on the amplitude of the rod response or its kinetics. Depolarizing currents reduced the amplitude of both peak and plateau components of the response to the same extent.

Effects of the cardiac glycoside ouabain: 0.1 mM ouabain was delivered to the retina causing a gradual depolarization and

depression in the amplitude of the light response. The initial peak of the response was reduced more than the plateau.

These preliminary results suggest that light not only reduces the permeability of the rod plasma membrane to sodium but also that other mechanisms are involved in the genesis of the rod light response (perhaps potassium conductance changes). Further, the experiments with extrinsic current suggest that the conductance of the rod plasma membrane may be voltage-dependent.

Significance to Bio-medical Research and the Program of the Institute: This project has significance primarily at the basic research level. Much is known about the ionic mechanisms involved in propagation of electrical signals along the nerve axons and in synaptic transmission. To date, no description of a primary sensory transducer has been presented which can account for the preliminary results described above. A comparison of the ionic specificities involved in the generation of the rod light response with the membrane properties of axons may provide fundamental insight into the basic mechanisms underlying neuron function.

Proposed Course of the Project: The experimental results just described were preliminary and as such are considered to be pilot studies to direct future experiments. These results must be repeated and expanded. Complete characterization of the relationship between ionic concentrations and membrane polarization and light response amplitudes will be determined. From these measurements, the ionic composition of the cytoplasm of the rod may be deduced. Further experiments performed with ouabain may provide answers to the question of an involvement of electrogenic pumps in the generation of the rod response.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01889-06 LNP
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PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on Agents Affecting Cell Proliferation and Differentiation in the Crystalline Lens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: L. von Sallmann
OTHER: P. A. GrimesResearch Neurologist
ChemistLNP NINCDS
LNP NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

Section on General Physiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01943-05 LNP
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PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (30 characters or less)

Spontaneous Retinal Degeneration in Osborne-Mendel Rats

NAME, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. von Sallmann	Research Neurologist	LNP NINCDS
OTHER:	P. A. Grimes	Chemist	LNP NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

Section on General Physiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02154-02 LNP
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PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Organization of Vertebrate Retinal Neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	P. O'Bryan	Senior Staff Fellow	LNP NINCDS
OTHER:	G. Bertrand	Visiting Fellow	LNP NINCDS
	W. Stewart	Staff Fellow	LEP NIAMDD

COOPERATING UNITS (if any)
Laboratory of Experimental Pathology
NIAMDD

LAB/BRANCH
Laboratory of Neurophysiology

SECTION
Section on General Physiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
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SUMMARY OF WORK (200 words or less - underline keywords)
This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBR (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02155-02 LNP								
PERIOD COVERED July 1, 1975 to June 30, 1976										
TITLE OF PROJECT (80 characters or less) Organization of a Molluscan Vestibular System										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0"> <tr> <td>PI:</td> <td>R. Fioravanti</td> <td>Visiting Scientist</td> <td>LNP NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>M. G. F. Fuortes</td> <td>Chief, LNP</td> <td>LNP NINCDS</td> </tr> </table>			PI:	R. Fioravanti	Visiting Scientist	LNP NINCDS	OTHER:	M. G. F. Fuortes	Chief, LNP	LNP NINCDS
PI:	R. Fioravanti	Visiting Scientist	LNP NINCDS							
OTHER:	M. G. F. Fuortes	Chief, LNP	LNP NINCDS							
COOPERATING UNITS (if any) None										
LAB/BRANCH Laboratory of Neurophysiology SECTION Section on General Physiology INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014										
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:								
SUMMARY OF WORK (200 words or less - underline keywords) This project has been terminated.										

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02157-02 LNP
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (60 characters or less) Sensory Receptors in Hermissenda Crassicornis		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	P. E. Detwiler	Staff Fellow LNP NINCDS
OTHER:	M. G. F. Fuortes	Chief, LNP LNP NINCDS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neurophysiology		
SECTION Section on General Physiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
SUMMARY OF WORK (200 words or less - underline keywords) This project has been terminated.		

Project Description:

Objectives: To investigate the role of calcium in the transduction process in the turtle photoreceptors.

Methods Employed: Cone responses were recorded intracellularly with glass microelectrodes in a perfused eyecup preparation. Internal or external concentration was varied, and the responses to various stimuli were recorded. A special perfusion system has been developed to change the external calcium concentration; the internal calcium concentration was varied by iontophoretic or pressure injection of a calcium chelating agent through the recording microelectrode.

To improve the speed and accuracy of data acquisition and storage, an on-line link-up with our PDP 11 computer system has been developed.

Major Findings: It was found that addition of EGTA to the external medium results in a depolarization of the membrane in the dark, a decrease of the amplitude of the responses to dim flashes, an enhancement of the amplitude and a slowing of the time course of the response to bright flashes. Low external calcium results in an increased membrane conductance, and this change can explain the depolarization of the cell in the dark and the modification of the amplitude of the response to both dim and bright flashes. Increased conductance, however, cannot explain the changes in the time course of the response. Lowering the internal calcium concentration results only in a decrease of the amplitude of the response. Experimental results are analyzed using the model developed by Baylor, Hodgkin and Lamb.

Significance to Bio-medical Research and the Program of the Institute: The purpose of this project is to advance our understanding of the excitation transduction process in photoreceptors.

Proposed Course of the Project: Studies will be continued to determine the role of the calcium in the transduction process. Since one of the investigators is returning to Switzerland, collaboration will be established between this laboratory and the laboratory in the Department of Physiology, Medical Faculty, University of Geneva.

Publications:

Fuortes, M.G.F.: Interactions and Feedbacks in the Turtle Retina. In Zettler, F. and Weiler, R. (Ed.): Physiology of the Retina. Springer-Verlag of Heidelberg. In press.

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Noise Analysis in Rods and Cones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Cervetto	Visiting Scientist	LNP NINCDS
OTHER:	F. Conti	Visiting Scientist	LNP NINCDS
	M. G. F. Fuortes	Chief, LNP	LNP NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

Section on General Physiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

SUMMARY OF WORK (200 words or less - underline keywords)

Noise analysis is performed on photoreceptor cells of vertebrates in light and darkness. It is found that noise decreases with illumination but the origin of the noise is as yet unclear.

Project Description:

Objectives: To clarify the elementary mechanisms leading to photoreceptor responses.

Methods Employed: Turtle or toad retinae are isolated and perfused. Low-resistance microelectrodes are inserted in the retina and their noise (N_L) is measured. Retinal rods and cones are then impaled and the noise measurement is repeated in light (N_L) and darkness (N_D). Difference spectra are then constructed of $N_D - N_L$ and $N_L - N_E$. It is thought that the results will provide useful information on the mechanisms responsible for the responses of rods and cones.

Major Findings: Since this project was initiated only a few months ago, the results obtained so far are preliminary. Present evidence indicates that in both rods and cones, noise is usually greater in darkness than in light. The results however are quite variable both in different cells and in one cell at different times. For this reason it appears doubtful that the noise recorded in these experiments is due to fluctuations in the number of ionic channels in the photoreceptor membrane.

Significance to Bio-medical Research and the Program of the Institute: Recent work on the retina has revealed new basic mechanisms of neuronal action. In photoreceptors, illumination evokes hyperpolarization and increased membrane resistance, suggesting that more "sodium channels" open in darkness than during illumination. These results, together with the kinetic properties of the response had led to the view that light causes the production of particles which block the sodium channels. Noise analysis should provide useful details on the mechanisms of this unconventional action.

Proposed Course of the Project: Noise analysis will be continued in photoreceptors and will be extended to other retinal cells. Voltage fluctuations are recorded from rods and cones in darkness or during constant illumination. The power spectrum of the fluctuations is analyzed in the hope that it will provide useful information on the elementary mechanisms of visual responses of vertebrate photoreceptors.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02230-01 LNP
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Computer Simulation of Photoreceptor Responses		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: J. Pochobradsky Visiting Fellow LNP NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neurophysiology		
SECTION Section on General Physiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.8	OTHER: 0.0
SUMMARY OF WORK (200 words or less - underline keywords) Theoretical equations which predict the <u>electrical response</u> of <u>turtle cones</u> to light stimuli are modified on the basis of experiments with <u>low calcium</u> . Main features of the existing theory are preserved, only some of its 19 parameters are changed and one parameter is added. Central points of the new <u>theory</u> are that blocking of light sensitive channels takes place even in darkness and that blocking particles are removed by calcium buffer <u>EGTA</u> .		

Project Description:

Objectives: To propose and test a theory of the electrical responses of turtle cones deprived of calcium.

Methods Employed: Hodgkin's theory of turtle cone responses was adapted to explain experiments done in this laboratory (project no. Z01 NS 02221-01 LNP). The responses were defined in terms of a set of differential and algebraic equations. These were solved numerically on a digital computer DEC-10. Programs were developed to allow simulation, least square fitting, changes of the model between runs, and plotting and tabulating the results. The language of choice was MLAB.

Major Findings: Electrical responses to light are attributed to changes of sodium current through the outer segment membranes. It is proposed that sodium channels are blocked by a blocking substance Z_1 whose production is triggered by light.

When the eyecup was perfused with calcium buffer EGTA, the saturating responses to bright flashes grew bigger due to the depolarization of the resting level. This was explained by the assumption that initial concentration z_{10} of Z_1 in darkness is normally greater than zero, and reduced by EGTA. An estimate of z_{10} was obtained from least square fitting of Michaelis-Menten type relationship to responses to steps. If all EGTA does is to reduce pre-existing z_{10} (say, indirectly by depleting the reservoir of its dark source), then a suitable background could compensate for it. Experiments show that it is not the case. Combination of EGTA and background can imitate normal peak height, but shortens time to peak. This result was, however, predicted when it was assumed that EGTA removes not only pre-existing Z_1 , but also that generated by light. Thus EGTA either speeds up conversion of Z_1 to Z_2 or drains it off via an independent leak. The latter was assumed. The analysis of responses to steps also supported this hypothesis.

Other modifications of the Hodgkin's model were tested. First, it was assumed that EGTA changes the rate of the reaction Y_5 to Z_1 . Parameter α^* assumed a role of concentration of extracellular Ca, while Y_5 was the light controlled permeability to Ca. Such model did not reproduce the change of the resting potential. Secondly, a suitable increase of G_1 alone (dark conductance of light sensitive channels) could mimic the increase of peak hyperpolarization after bright flash and its decrease after dim flash, but did not reproduce the change of time to peak.

The mentioned findings are compatible with the hypothesis that calcium is the blocking substance.

Significance to Bio-medical Research and the Program of the Institute: This project may contribute to better understanding of excitation of vertebrate photoreceptors. More may be learned about complex role of Ca in nerve system and in retina in particular.

Proposed Course of the Project: To conclude this work on turtle cones, new experiments with high Ca and EGTA injection will be performed and analyzed. Later, the role of calcium in toad rods will be studied.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01895-06 LNP	
PERIOD COVERED July 1, 1975 to June 30, 1976					
TITLE OF PROJECT (80 characters or less) Spectroscopic Studies on Vertebrate Visual Pigments in Cells and in Solutions					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT					
PI: F. I. Harosi		Senior Staff Fellow		LNP NINCDS	
OTHER: W. K. Stell		Associate Professor		UCLA, Calif.	
M. A. Ali		Professor, Dept. Biology		U. Montreal	
H. J. Wagner		Demonstrator		U. Ulm, F.R.G.	
C. Sandorfy		Professor, Dept. Chemistry		U. Montreal	
H. Shichi		Chemist		LVR NEI	
COOPERATING UNITS (if any)					
University of California		Los Angeles, California			
University of Montreal		Montreal, Canada			
University of Ulm		Federal Republic of Germany			
LAB/BRANCH					
Laboratory of Neurophysiology					
SECTION					
Section on Neuronal Interactions					
INSTITUTE AND LOCATION					
NINCDS, NIH, Bethesda, Maryland 20014					
TOTAL MANYEARS:		PROFESSIONAL:		OTHER:	
2.0		1.8		0.2	
SUMMARY OF WORK (200 words or less - underline keywords)					
<p>The purpose of this project is to study the molecular nature of visual pigments. The topics of interest are: 1) <u>Spectroscopic properties</u> of visual pigments by <u>computer analysis</u> of absorption spectra; 2) Correlations between cone <u>cell morphology</u> and visual pigment content in <u>goldfish</u>; 3) Correlations between cone morphology, pigment type, habitat, and evolutionary trends among the <u>bony fishes</u>; 4) Band structure in visual pigment absorption spectra; 5) Roles of <u>phospholipids</u> in solubilized <u>cattle rhodopsin</u>.</p>					

Project Description:

Objectives: It is the long range purpose of this project to study the chemical and structural nature of visual pigments and the photoreceptors containing them pertaining to photic excitation. Topics of current study are: 1) Spectroscopic properties of vertebrate visual pigments by computer analysis of absorption spectra obtained from single photoreceptors by microspectrophotometry and by spectrophotometry of pigments in solution; 2) Correlation of cone cell morphology with visual pigment content by histologic examination and microspectrophotometry of the goldfish retina (with W.K. Stell); 3) Inter relationships between photoreceptor morphology, cone and rod connectivity with second order neurons, visual pigment content as to type, animal habitat, and evolutionary trends in bony fishes by light and electron microscopy and microspectrophotometry (with M.A. Ali and H.J. Wagner); 4) Band structure of visual pigment and related absorption spectra by theoretical spectroscopic methods (with C. Sandorfy); 5) Roles of phospholipids in solubilized cattle rhodopsin by combining chemical treatments of extracts with spectroscopic and post-photolized kinetic measurements (with H. Shichi).

Methods Employed: The main experimental method is absorption spectroscopy of visible and ultraviolet light by single cells and by microscopic and usual volumes of solutions. Other methods are: histology for light and electron microscopy, chemical separations and purifications (chromatography), computer analysis and modeling and theoretical spectroscopy.

Major Findings: In the course of the computer analysis of experimental spectra, it was discovered that the total area (or the sum of the areas of the three major components) is about the same in the molar extinction vs. wavenumber spectra of the prosthetic groups and of the corresponding visual pigments. Thus, 11-cis dehydrorétinal forms a spectroscopic continuum with all the porphyropsins and 11-cis retinal another continuum with all the rhodopsins. The apparent spectral transformations in both series were described as parameter variations in the sum of three Gaussian functions which were obtained by curve-fitting to a principal set of experimental spectra. The analytic functional descriptions so obtained permit the accurate generation of all vertebrate visual pigment spectra, replacing previously existing nomograms and the hypothesis of invariance of oscillator strength of the main bands.

Significance to Bio-medical Research and the Program of the Institute: The accurate description of absorption spectra is fundamental to physiologists and psychophysicists concerned with vision. The chemical nature of rhodopsin and the band structure

of its absorption spectrum are fundamental aspects of a detailed understanding of its function, namely, how light is capable of initiating a process of cell excitation.

Proposed Course of the Project: It is to be discontinued as the principal investigator's appointment terminates on June 29, 1976.

Publications:

Harosi, F.I.: Microspectrophotometry: the technique and some of its pitfalls. In Ali, M.A. (Ed.): Vision in Fishes: New Approaches in Research. New York, Plenum, 1975, pp. 43-54.

Harosi, F.I.: Linear dichroism of rods and cones. In Ali, M.A. (Ed.): Vision in Fishes: New Approaches in Research. New York, Plenum, 1975, pp. 55-65.

Harosi, F.I.: Absorption spectra and linear dichroism of some amphibian photoreceptors. J. Gen. Physiol. 66: 357-382, 1975.

Stell, W.K. and Harosi, F.I.: Cone structure and visual pigment content in the retina of the goldfish. Vision Res. In press.

Harosi, F.I.: Spectral relations of cone pigments in goldfish. J. Gen. Physiol. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02152-02 LNP
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PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Neural Connections in the Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Kolb	Senior Staff Fellow	LNP NINCDS
OTHER:	E. V. Famiglietti, Jr.	Post-doctoral Fellow	LVR NEI
	R. Nelson	Staff Fellow	LVR NEI
	P. Gouras	Head, Section on Physiology	LVR NEI

COOPERATING UNITS (if any)

National Eye Institute
Bethesda, Maryland

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

Section on Neuronal Interactions

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

SUMMARY OF WORK (200 words or less - underline keywords)

In cat retina the rod and cone pathways remain segregated into separate neuronal types in the vertical pathways. In addition, the two cone pathways remain segregated in cat retina both by contacts with the cones at the OPL and by segregating their input to different subtypes of the same ganglion cell class in the IPL. Direct mixing of rod and cone information could occur, however, at the gap junctions between the cones and rods at the OPL by interreceptor contacts and at the AII amacrine and invaginating cone bipolar gap junctions in the IPL. Horizontal and amacrine cells must also be responsible for mixing rod and cone information spatially at the plexiform layers, while the interplexiform cell probably provides a feed-back loop of mixed rod/cone signals from the inner to the outer plexiform layer.

Project Description:

Objectives: To attain an understanding of the neural circuitry of the vertebrate retina.

Methods Employed: The retina of the vertebrate eye (cat or monkey) is studied by 1) light microscopy of Golgi-impregnated material, 2) electron microscopy of Golgi-impregnated material, 3) ultra-thin serial sectioning techniques and 4) correlations with micro-electrode recordings and Procion marking of neurons.

Major Findings: In the retina of the cat the synaptic relationships of the neurons in the two plexiform layers has been studied. The rod and cone pathways remain segregated by vertical neuron chains -photoreceptors-bipolars-(amacrines)-ganglion cells, but mixing of rod and cone signals appears to happen by means of electrical or gap junctions at the photoreceptor level and in the two plexiform layers. Thus, in the outer plexiform layer (OPL) cone pedicles have basal processes which project to touch neighboring rods by means of minute gap junctions. Physiological studies indicate that these junctions are concerned with mixing rod and cone signals (Nelson, 1976). The rods do not appear to have any morphological counterpart of electrical junctions in the retina of the cat.

In the inner plexiform layer (IPL) the rod and cone channels remain segregated by stratification of their axon terminals in different portions of the neuropil. Thus rod bipolar cells end deep in the IPL and do not contact ganglion cells directly but use instead an internuncial amacrine (AII) to pass information to certain ganglion cells. The rod pathway is thus disynaptic to ganglion cells in contrast to the monosynaptic direct cone pathway. However, the two cone bipolar types in cat retina (flat and invaginating) differ in contacting exclusively different subtypes of ganglion cell of the same class. Flat bipolar cells synapse only on ganglion cells branching in sublamina A, i.e. classes Ia, IIa and IIIa, while invaginating cone bipolar cells connect only to ganglion cells in Ib, IIb and IIIb which branch only in sublamina B of the IPL.

Rod and cone signals do eventually converge at the ganglion cells. In fact we know from physiological experiments that all ganglion cells in cat retina have rod and cone input. A gap junction between the AII amacrine cell in the rod pathway and the invaginating cone bipolar is a means whereby rod and cone information is mixed in the IPL before the ganglion cells. In addition some amacrines must be interlinked across the retina and be contacting both rod and cone bipolar terminals in the IPL before synapsing on the ganglion cells. One amacrine cell type, the interplexiform cell has dendrites in both plexiform layers and

appears to be concerned with feeding back information from the IPI to primarily bipolar cell dendrites in the OPL. Thus, this type is also mixing rod and cone pathways although in a diffuse manner integrating units over a wide area of the retina.

Significance to Bio-medical Research and the Program of the Institute: Studies of the structure of the retina will provide a valuable understanding of the connections of the cells within the retina and will in all probability relate to neural circuitry elsewhere in the CNS. Many programs of this Institute are concerned with the physiology and marking of single retinal neurons and thus knowing the morphology and connectivity of these same neurons is essential for our further understanding of visual events.

Proposed Course of the Project: It is proposed to continue the study of the structure of the retina to obtain further insights into the relevance of structure to function in the CNS.

Publications:

Nelson, R., Lutzow, A., Kolb, H. and Gouras, P.: Horizontal cells in cat retina with independent dendritic systems. Science 189: 137-139, 1975.

Boycott, B.B., Dowling, J.E., Fisher, S.K., Kolb, H. and Laties, A.M.: Interplexiform cells of the mammalian retina and their comparison with catecholamine-containing retinal cells. Proc. Roy. Soc. 191: 353-368, 1975.

Kolb, H., Famiglietti, E.V. and Nelson, R.: Neural connections in the inner plexiform layer of the cat's retina: Third Symposium on "The Structure of the Eye." Jap. J. Ophthal. In press.

Nelson, R., Kolb, H., Famiglietti, E.V. and Gouras, P.: Neural responses in the rod and cone systems of the cat retina: Intracellular recordings and Procion stains. Invest. Ophthal. In press.

Kolb, H. and Famiglietti, E.V.: Rod and cone pathways in the retina of the cat. Invest. Ophthal. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01690-08 LNP
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PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Rapid Scanning Microspectrophotometry in Visual Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. G. Smith, Jr.	Medical Officer (Res)	LNP NINCDS
OTHER:	J. Oldak	Graduate Student	Univ. of Md.
	T. Colburn	Electronic Engineer	TD, NINCDS

COOPERATING UNITS (if any)

Dept. of Biomedical Engineering University of Maryland	Section on Technical Development NIMH, NINCDS
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LAB/BRANCH

Laboratory of Neurophysiology

SECTION

Section on Sensory Physiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

1.3

PROFESSIONAL:

1.3

OTHER:

0

SUMMARY OF WORK (200 words or less - underline keywords)

A rapid scanning microspectrophotometer has been designed and constructed which allows absorption spectra to be measured between 350 and 650 nm at a speed of 0.6 msec per wavelength band (10 nm). Experiments on frog (Rana pipens) are in progress to measure the effects of several variables on visual pigment (rhodopsin) kinetic changes. These variables include pH, temperature, divalent cations, presence and absence of pigment epithelium and degree of attachment of rod outer segments.

Project Description:

Objectives: The objective of this research is the development of rapid scanning microspectrophotometric techniques for investigation of the chemical kinetics of excitable cells. Although these techniques are being developed to study the molecular steps coupling photoexcitation of visual pigments to the excitation of the electrical response of the cells, their more general application to cellular physiology will also be considered.

Methods Employed: The microspectrophotometer samples transmittance at spectral wave bands between 350 and 650 nm, either sequentially or in random order, at rates of 600 microseconds per sample point. The system uses a rapid scan monochromator in which the entrance slit has been replaced by the image of a cathode ray tube face.

Major Findings: As a result of the improvements in and further testing of the rapid scanning microspectrophotometer, detailed in last years Annual Report, the instrument is currently operational and biological experiments have been underway since June 1975. These experiments have been performed on retinas from the frog, Rana pipiens. As a further test of the machine and to attain competence with the biological preparation, the initial experiments attempted to reproduce experiments previously reported in the literature by other laboratories. These earlier experiments were performed on slow microspectrophotometers and hence were concerned with the slow kinetics of visual pigments. Specifically, our initial experiments dealt with the reported effects of pH on visual pigment spectra and kinetics. Many experiments were consistent with those in the literature; however, in all pH categories, there were discrepancies in the data which could not be ascribed to "failures" or "artefacts." Current experiments in progress are attempting to discover those preparation variables (degree of attachment of the retinae to the pigmented epithelium, connected vs. separated rod outer segments, preparation oxidation, divalent cations, etc.) which can account for the variation observed.

Significance to Bio-medical Research and the Program of the Institute: This project should provide the specific instrumentation and techniques for the measurement and analyses of the transduction steps which couple the stimulus to the electrical changes in the excitable membrane of photoreceptors. It will also provide a useful tool for similar study of other molecular systems functioning within living cells, for which an increasing need has developed.

Proposed Course of the Project: The aforementioned studies to account for experimental variation will be undertaken. In addition, since our experiments have shown that the main time-limitation in the microspectrophotometer's performance is in the energy and hence the duration of the light pulse required to bleach visual pigment adequately, alternative methods are being explored to achieve a brighter bleaching light.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02019-04 LNP
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PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Electrophysiology of Simple Cellular Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. G. Smith, Jr.	Medical Officer (Res)	LNP NINCDS
OTHER:	J. Barker	Medical Officer	BB NICHHHD
	K. Futamachi	Guest Worker	LNP NINCDS
	T. Akaike	Assistant Professor	Univ. of Tokyo
	G. Fischbach	Associate Professor	Harvard Univ.
	J. Fukuda	Assistant Professor	Univ. of Tokyo
	W. Sheriff	Computer Specialist	TD, NINCDS
	M. Henkart	Staff Fellow	NICHHHD

COOPERATING UNITS (if any)

Behavioral Physiology Branch, NICHHHD	Bethesda, Maryland
Department of Pharmacology	Harvard Medical School
Department of Physiology	University of Tokyo

LAB/BRANCH
Laboratory of Neurophysiology

SECTION
Section on Sensory Physiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: <u>4.5</u>	PROFESSIONAL: <u>4.4</u>	OTHER: <u>0.1</u>
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SUMMARY OF WORK (200 words or less - underline keywords)

Electrophysiological experiments with microelectrodes have been performed on chick muscle cells grown in tissue culture and on identified neurons of molluscs. Using the voltage clamp technique the membrane conductances underlying slow membrane potential responses in these preparations have been investigated. Studies on the muscle cells have concentrated on the voltage and time dependent characteristics of the chloride conductance underlying the chloride spike and on the relationship of this spike to the caffeine contracture. Studies on the molluscan neurons have concentrated on the membrane conductances underlying both the spontaneous pacemaker activity and the epileptiform activity found in these neurons. These experiments have focused mainly on the role of the cation (Na^+ , K^+ , Ca^{2+}) conductances in the generation and control of both types of electrical activity. In addition, the effects and the mechanisms of action of various drugs and hormones on such activity have been investigated.

Project Description:

Objectives: The objective of this research is to gain insight into the membrane mechanisms which are responsible for the activity of single cells.

Methods Employed: The electrical properties of single cells are recorded with intracellular microelectrodes and analyzed under a variety of experimental conditions. The technique of voltage-clamping the membrane potential is the most important method employed in these experiments. This technique requires the penetration of single cells with two microelectrodes, one for monitoring the membrane potential, the other for supplying the current necessary to clamp the membrane potential at the desired level. Using this technique the role of the various ionic conductances responsible for the electrical responses of the membrane can be studied. In addition, the effects of changing the intracellular and/or extracellular ions and of adding pharmacological agents on membrane properties can be evaluated.

Because of the necessity of taking many measurements from each experiment, the data is often recorded on magnetic tape for later analysis with a PDP-11 computer (see below).

The anatomical properties of the cells are studied with conventional light and electronmicroscope techniques.

Major Findings: This project involves the investigation of the electrical characteristics of nerve and muscle tissue, with particular emphasis on slow membrane processes. This past year most investigations have been on chick muscle cells grown in tissue culture and on neurosecretory cells of molluscs.

1. Tissue cultured muscle cells. A number of electrical properties of the chick muscle cells have been identified and studied during the course of development of these cells from their immature, myoblastic stage to an apparently "mature" stage. One particularly interesting electrical response in these cells is a slow "spike" which is associated with a slow contraction of the muscle. This spike appears quite early in development and changes with maturation. The results of electrophysiological investigations indicate that it is generated by a voltage- and time-dependent change in the muscle membrane's chloride permeability. Thus, the chick muscle has three types of spikes: 1) a conventional, fast sodium spike, 2) a slower calcium spike and 3) a very slow chloride spike.

In order to have a technically manageable preparation for the voltage clamp experiments, the muscle cells have been grown in

10-20 nanomolar colchicine, which results in a spherical, isopotential cell (a myosac) with apparently normal membrane electrophysiological properties. The electronmicrographic studies indicate that colchicine prevents the formation of microtubules and hence the elongation of the cell into a tubular fiber. These myosacs have all the spikes (sodium, calcium, and chloride) of the normal muscle, as well as sensitivity to acetylcholine. Moreover, they can contract spontaneously or following stimulation.

During this past year the experiments, which allow characterization of the voltage-dependent chloride conductance that underlies the slow chloride spike, were completed and a paper dealing with this data is in preparation. In addition studies were undertaken to compare and contrast the electrical and mechanical events associated with the electrically-evoked chloride spike and the chemically-evoked caffeine contraction. Another matter of interest has been the characterization of the resting chloride conductance and permeability of the tissue cultured muscle. We have been confronted, in this regard, with a vexing problem, namely, that the original data obtained by Dr. Fukuda cannot be confirmed in more recent experiments by Dr. Akaike. We have examined carefully all the relevant variables we know of but have been unable to discover the basis of these discrepant results. We plan to have Drs. Fukuda and Akaike work together on this important problem in an attempt to resolve the issues.

2. Molluscan neurosecretory cells. One aim of the study of the snail neurosecretory cells is an understanding of the membrane mechanism underlying the bursting, pacemaker activity (BPA). Voltage-clamp analysis has shown that bursting cells have an N-shaped, steady-state current-voltage (I-V) curve. The cell has no "resting" potential but oscillates between the two positive slope regions of the I-V curve.

As previously reported, ionic studies have shown that the two positive slopes of the I-V curve result from high potassium conductances, which are voltage- and time-dependent. The positive slope at large negative potentials is due to anomalous rectification while that at small negative and positive potentials is due to other potassium conductances. The negative slope region of the N-shaped curve is due to a voltage-dependent, but apparently time-independent sodium conductance. Previously, a qualitative model of the membrane mechanisms underlying spontaneous pacemaker or oscillatory activity in molluscan neurosecretory cells was developed and published. Central to this model are the differences between the voltage, and time-dependencies of the sodium conductance and of the potassium conductances.

During this past year most of the experiments were devoted to developing and using experimental paradigms which provide some separation and thereby better measurement of the several voltage and time dependent conductances. The experiments have been successful but the analysis of the data by hand has proved extremely laborious and time consuming. Since we have lost access to the PDP-12 computer previously used to analyze voltage-clamp data, new data analysis programs are currently being developed for use on the PDP 11/10 computer recently obtained by our laboratory. Mr. William Sheriff, of the Section on Technical Development, is collaborating with us in this effort. When the data in hand are analyzed and the characteristics of the cell's membrane conductances documented, the result should be a fuller account of such conductances than heretofore possible and the techniques developed should have a wider utility than our own specific experiments.

As reported last year and recently published, we have found that certain polypeptides, similar to and including vasopressin, significantly increase bursting pacemaker activity in molluscan neurosecretory cells. Using our new experimental paradigms (see above), provisional results indicate that these polypeptides may exert their effects primarily by an action on the voltage dependent potassium conductances of these neurons. Results from other laboratories on other neuronal systems suggest that our findings may be of interest to disciplines other than the neurophysiology of invertebrates (see below).

During this past year, Dr. Kin Futamachi joined our laboratory as a post-doctoral fellow. Since arriving Dr. Futamachi has learned the techniques necessary to study snail neurons with the voltage-clamp. In addition, he has collaborated in the development of a new voltage clamp system for his own use. By incorporating several electronic techniques novel to voltage clamping, Dr. Futamachi has a system superior to those currently available.

Dr. Futamachi brings to our research program considerable experience in the electrophysiological study of epileptogenic neurons in experimentally induced epilepsy. He intends to investigate, with the voltage clamp technique, vertebrate neurons, grown in tissue culture, which are known to have electrophysiological properties similar to epileptogenic neurons found in vertebrate neurons *in vivo*. His goal is to determine the membrane and/or synaptic mechanisms by which neurons become epileptogenic. This work will be done in collaboration with Dr. Phillip Nelson's group of NICHD.

During his own experiments with snail neurons, and while he was gaining experience with the voltage-clamp technique, Dr. Futamachi discovered that certain experimental maneuvers cause otherwise normal cells to produce a variety of electrical activity

qualitatively similar to that found in vertebrate epileptogenic neurons. These maneuvers include alteration of the divalent cations in the extracellular fluid. Currently Dr. Futamachi is investigating the mechanisms underlying these phenomena, which were entirely unexpected. In addition, he plans to investigate the action of pharmacological agents on this epileptogenic-like activity. These agents include both those known to cause or enhance epilepsy as well as those known to be of therapeutic benefit in clinical and experimental conditions. Dr. Futamachi's results with the technically more manageable invertebrate neurons will be useful in planning his experiments on vertebrate neurons.

Significance to Bio-medical Research and the Program of the Institute: In the few years that this research project has been in existence, a number of scientific problems have been investigated which are not only of interest on their own right but also may be of wider importance to biomedical science. Such potential importance supports the contention, held by many biologically-oriented medical scientists, that the judicious selection of a experimentally manageable preparation, which possesses a phenomena of some general interest, is the most likely to yield meaningful and potentially significant results.

Our experiments with molluscan neurons illustrate this potential importance in several different areas.

In the first place, our results and our model of spontaneous pacemaker activity may be related to similar activity found in other excitable tissue such as heart muscle and some vertebrate central neurons system neurons. Secondly, similar considerations may apply to our recent finding of epileptogenic-like activity in molluscan neurons with respect to experimental and clinical epilepsy. Furthermore, in both of these areas (pacemaker activity and epileptogenic activity), the use of these preparations to assess the mechanisms of action of pharmacological agents may prove useful, since experiments can be done with these preparations that are extremely difficult or impossible to perform in higher species.

In addition, our results on the effects and mechanism of action of polypeptides, like vasopressin, on molluscan neurons has assumed a potential importance quite unexpected at the time the experiments were undertaken. Recently a number of scientists in neurophysiology, neuropharmacology and psychopharmacology have focused their attention on the effects and mode(s) of action of a number of peptides and polypeptides on neurohumoral and on exclusively neuronal systems in various species. Currently, there is considerable controversy over whether these compounds act like conventional neurotransmitters, such as acetylcholine, or whether they have some "other" mechanism of action. Our results

with molluscan neurons demonstrate that one polypeptide, vasopressin, has an "other" mode of action, which is quite unlike that of conventional transmitters.

While our experiments with tissue cultured muscle has produced results of theoretical and specific scientific interest, it is unclear how they may be related to other areas of biomedical science. For example, we have yet to ascertain what role, if any, certain phenomena, like the chloride spike, play in growth and maturation of skeletal muscle or whether they are merely atavistic expressions of the biogenic law. Moreover, exactly how and to what purpose they are merely transient expressions of the myocytes' genetic apparatus are unknown.

Proposed Course of the Project: We proposed to continue our analyses of the several voltage- and time-dependent conductances found on molluscan neurons, using the experimental methods we have developed. These analyses should be performed more efficiently when programs for the PDP 11 become available. Once the characteristics of these molluscan neuron membranes have been adequately documented, such knowledge will be the basis for studies both of the epileptogenic activity in these neurons as well as the mode of action of pharmacological agents of interest. Such agents include those which are known to affect pacemaker activity and those which affect epileptogenic activity. These studies, in turn, should be useful in our planned investigations of vertebrate tissue cultured neurons.

The future course of our laboratory's research on tissue cultured muscle cells is uncertain. As mentioned, Drs. Fukuda and Akaike will attempt to resolve the question of such cells' resting chloride permeability; but subsequently their research activities will probably proceed independently of our lab. With Dr. Akaike's recent departure, no one is currently continuing research on tissue cultured muscle in our laboratory, despite attempts to locate a suitable replacement. For example, a recent graduate from the Illinois Institute of Technology, Dr. Donna Gruol, applied to our laboratory to work, in collaboration with Dr. Phillip Nelson's group, on a very interesting problem concerning normal and dystrophic muscle cells grown in tissue culture. Her proposed project seemed especially attractive because it dealt with a scientific problem of interest in its own right and also had obvious potential relevance to other, including clinical, areas of biomedical science, like muscular dystrophy. Because of Institute and laboratory problems with position allocations, we did not have a laboratory slot to offer Dr. Gruol. We had, therefore, to make her acceptance by the laboratory provisional upon her obtaining a postdoctoral fellowship from the NIH Extramural Program. As a result of insufficient and uncertain funding in that program, Dr. Gruol was not awarded a fellowship.

Until some satisfactory arrangement can be found, this part of our research program will be regrettably suspended.

Publications:

Fukuda, J.: A voltage clamp study of currents of spherical muscle cells grown in tissue culture. Nature 257: 408-410, 1975.

Barker, J.L. and Smith, T.G., Jr.: Peptide regulation of neuronal membrane properties. Brain Res. 103: 167-170, 1975.

Fukuda, J., Henkart, M.P., Fischbach, G.D. and Smith, T.G., Jr.: Physiological and structural properties of colchicine treated chick skeletal muscle cells grown in tissue culture. Dev. Biol. 49: 395-411, 1976.

Fukuda, J., Fischbach, G.D. and Smith, T.G., Jr.: A voltage clamp study of the sodium, calcium and chloride spikes of chick skeletal muscle cells grown in tissue culture. Dev. Biol. 49: 412-424, 1976.

Annual Report
July 1, 1975 thru June 30, 1976
National Institute of Neurological Communicative
Disorders and Stroke
Laboratory of Biophysics
William J. Adelman, Jr., PhD, Chief

INTRODUCTION

The research program of the Laboratory of Biophysics is concerned with investigating molecular and cellular mechanisms responsible for excitation, membrane potentials, the generation of the nerve impulse, and synaptic activity. This program involves the study of natural membranes and artificial membrane systems. The laboratory is concerned with the biophysical basis for the functioning of simple nervous systems and with uncovering the cellular basis for such integrative neural functions as behavior and learning. The laboratory makes wide use of physical and chemical techniques, on-line and off-line digital computers and a variety of applied mathematical methods. During the period of this report the laboratory underwent a major reorganization. Two units and three sections were established. One of these units was established on a year-round basis at the Marine Biological Laboratory in Woods Hole, Mass. The Woods Hole Unit is composed of 2 sections; the Section on Neural Membranes and the Section on Neural Systems. The Bethesda unit of the laboratory was formed into a Section on Molecular Biophysics. The following advantages were seen in establishing a laboratory at Woods Hole:

(1) For a number of years the Laboratory of Biophysics conducted research on the squid giant axon during summers at the Marine Biological Laboratory. Since squid are available in Woods Hole in adequate numbers from April 15 thru December 1, the data collection period for this research by the Laboratory of Biophysics scientists would be greatly extended.

(2) One of the laboratory's more recent major research efforts has been the study of integrated functions of simple nervous systems in certain marine species. The superb animal collection and survey facilities available at the Marine Biological Laboratory permit numerous comparative studies of neural systems, axons, receptors, etc. found in these marine organisms.

(3) One aspect of this program has been the correlation of animal behavior with neural function. The Marine Biological Laboratory (MBL) possesses highly suitable animal behavior and mariculture facilities which will greatly facilitate these studies.

WOODS HOLE UNIT OF THE LABORATORY OF BIOPHYSICS:

Section on Neural Membranes.

The Section on Neural Membranes has been concerned with characterizing, in as much detail as possible, the model nerve fiber formed by the squid giant axon. Intracellular, membrane, and extracellular components of this

total system are studied. The primary thrust has been the examination of sodium and potassium channels in the axon membrane. These studies have been correlated with findings made on artificial membrane model systems such as the channel aggregation model, and with kinetic model schemes such as the energy barrier - ion site model and the specific ion site (SIS) model. Extensive studies are being carried out on the makeup of the intracellular neural cytoskeleton, its protein structure and the relation of this cytoplasmic structure to important values related to nerve impulse conduction as the axoplasmic resistance and viscosity. As the external Schwann sheath has been shown to influence the behavior of the axon, modifying its information processing in a non-synaptic manner, this sheath has been subject to an extensive electrophysiological and electronmicrograph analysis. Models derived from these studies have provided an anatomical/physiological basis for such important entities as the Frankenhaeuser-Hodgkin space and the series resistance. The section has also been concerned with pioneering and developing new methods and techniques for the study of neural phenomena.

The experimental program of the section has made the following advances. Experiments on ion interactions in open potassium channels of the squid giant axon membrane have provided information about the energy barriers crossed by ions entering these channels and about the nature of ion binding sites within these channels. An extensive review on competition, saturation and inhibition of membrane ion currents in nerve, muscle and bilayer systems has given breadth and scope to the ion interaction studies in squid nerve membrane.

The "inertia" seen previously in potassium channels following large brief depolarizations was confirmed and the study was extended. Extensive calculations were performed to examine predictions of the Hodgkin-Huxley model under similar circumstances. These calculations indicated a qualitative divergence between experimental results and Hodgkin-Huxley predictions. In contrast, the experimental observations were consistent with predictions of the Baumann-Mueller model. In another study, a specific ion-site interaction scheme using enzyme kinetic methods was applied to describe the inhibitory effect of external potassium ions on inward sodium currents in the squid giant axon.

In a collaborative effort with Carnegie-Mellon University, squid and *Myxicola* axon neural filaments were examined as to their structure and biochemical makeup. Of particular interest were intermediate sized neural filaments of 10 nm diameter. 10 nm filaments were also found in smooth muscle cells of chicken gizzard. Evidence was obtained indicating that these were related to a protein having a molecular weight of 160,000 Daltons. The proteins from squid and *Myxicola* axoplasm were shown to coelectrophorese with the protein from the chicken gizzard muscle. As these intermediate filaments are found in many eucaryotic cells, such filaments have been taken by many workers to be an important part of the cytoskeleton. The section has continued its studies on the Schwann sheaths enclosing the giant axons of both squid and *Myxicola*. On the basis of montage electronmicrographs, it has been possible to derive an anatomical reconstruction of the Schwann sheath. Using this model, the resistance in series with the excitable membrane of the giant axon has been calculated to be about $0.6 \Omega \text{ cm}^2$. The importance and significance of the Schwann sheath in non-synaptic modulation of neural activity was communicated to the Society for Neuroscience in an invited symposium lecture

at the 1975 Annual Meeting in New York City. Analysis of impedance measurements made on the squid giant axon has indicated that these are consistent with the anatomy of the Schwann sheath as revealed by electronmicroscopy. Calculated values for the series resistance from both the anatomical model and the impedance measurements are consistent with biophysical and physiological measurements of the value of the series resistance.

One of the major projects and services rendered by the Laboratory of Biophysics has been the development of data acquisitions systems for obtaining excitable membrane conductance data in a form that can be analyzed by digital computing machines. The latest advance in this area was the establishment in Woods Hole of a new system making use of a PDP-11/10 digital computer with dual floppy disks, 9-track read-write magnetic tape, graphics terminal, and hard-copy units as peripherals. This system is now being used in the study of ion interactions in sodium and potassium channels in the squid giant axon by the Section on Neural Membranes. The general configuration of this unit and its software operating system has been used as a model by the Section on Technical Development of NINCDS/NIMH for designing and constructing systems to be used by other laboratories in the institutes. New bridge techniques for measuring impedance of giant axon membranes and Schwann sheaths have been developed. Early tests of an "underground" bridge indicate it will be a great improvement over more conventional bridge techniques.

The above highlights of the program of the Section on Neural Membranes are examples of its integrated approach to understanding the role of the excitable membrane in neural phenomena, not only in terms of ion channels, per se, but as this role is influenced by intracellular and extracellular structures as well.

Section on Neural Systems

The principal objective of the section is to study the mechanisms by which simple neural networks process information with particular emphasis on mechanisms of learning. The nervous system of Hermisenda crassicornis has proven to be an excellent model for information processing at several levels: sensory transduction by photoreceptors and hair cells, analysis of synaptic circuitry, changes in synaptic circuitry produced by conditioning paradigms administered to intact animals as well as to isolated nervous systems, membrane properties modified by conditioning, identification of critical developmental stages for the neural networks studied as well as stages critical for learning.

Although in the past many neural preparations have been studied with similar goals, none have been understood with the precision necessary to ask questions about neural network function on a cellular level. Because a cell by cell analysis has been possible for the visual and vestibular pathways of Hermisenda, cellular principles of network function have emerged. Presynaptic inhibition, for example, has now been shown to be responsible for persistent changes of network function.

The approach of the Section, because of its residence at the Marine Biological Laboratory, can be broad both in the scientific methods employed

as well as in its spectrum of biological interests. A large number of marine forms at different evolutionary stages, are available for comparative studies. Flowing seawater holding facilities make possible long-term behavioral and correlated electrophysiological and biochemical analyses. Mariculture of successive Hermisenda generations is also becoming a reality. Among the techniques employed thus far are the following: simultaneous intracellular recording from multiple neural elements, paired stimulation of the visual and vestibular pathways using a rotating table, iontophoresis of fluorescent dyes and electron dense materials, automated behavioral monitoring, subcellular fractionation, protein phosphorylation analysis and uptake of neurotransmitter precursors.

Taking a broad approach in pursuit of its primary objective, the section has brought together a number of scientists highly trained in a variety of disciplines: electrophysiology, animal behavior, biochemistry, histology and embryology. This breadth of scientific technique and biological perspective are already yielding important results. These include cellular mechanisms of conditioning, non-synaptic interneuronal communication, transducer properties of hair cells, electromicroscopical identification of synaptic endings, neurochemistry of network elements, and veliger physiology.

Bethesda Unit of the Laboratory of Biophysics

Section on Molecular Biophysics

The Section on Molecular Biophysics is concerned with uncovering mechanisms responsible for controlling ionic current flow across axonal membranes and the influence of various chemical and pharmacological agents on these ionic movements. The section also examines acetylcholine activated ionic channels in post-synaptic membranes. Electrical fluctuations are measured in tissue-cultured skeletal muscle cells in order to determine unit conductances of excitable ionic channels. Ionic channels in lipid bilayers are studied. In particular, hemocyanin channels and EIM (excitability-inducing material of bacterial origin) are examined both as to their negative resistance characteristics and their single channel opening and closing kinetics. A number of mathematical model systems are studied. These include models for the phenomena of anodal break excitation in nerve, the optimal motor control of muscular motion, the basis for periodic bursts of impulses in bursting pacemaker neurons. One aspect of the mathematical studies is the development of interactive computer programs for solving nerve equations.

Another program of the section involves a study of the influence of drugs and physical variables, such as pressure on the voltage dependence of ionic channels in axonal and synaptic membranes. The action of drugs, such as yohimbin, and toxins, such as Batrachotoxin, are examined in detail. Another effort of the section is to determine how and to what extent the perineurium is involved in the maintenance and regulation of the ionic and metabolic environment of the axons of peripheral nerves. Various mechanisms for ion permeability across the perineurium are of interest in this study, and efforts are made to choose among alternative possibilities.

NATIONAL SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do not use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER <div style="text-align: center; font-weight: bold;">201-NS 01950-05 LB</div>																
PERIOD COVERED <div style="text-align: center; font-weight: bold;">July 1, 1975 to June 30, 1976</div>																		
TITLE OF PROJECT (80 characters or less) <div style="text-align: center; font-weight: bold;">Analysis of Excitable Membrane Characteristics by Means of Computer Controlled Voltage Clamp Techniques and Impedance Measurements.</div>																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																		
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%; vertical-align: top;">PI:</td> <td style="width: 35%; vertical-align: top;">W.J. Adelman, Jr.</td> <td style="width: 15%; vertical-align: top;">Chief</td> <td style="width: 35%; vertical-align: top;">LB NINCDS</td> </tr> <tr> <td style="vertical-align: top;">Other:</td> <td style="vertical-align: top;">K.S. Cole</td> <td style="vertical-align: top;">Res. Biophysicist</td> <td style="vertical-align: top;">LB NINCDS</td> </tr> <tr> <td></td> <td style="vertical-align: top;">R.J. French</td> <td style="vertical-align: top;">Visiting Fellow</td> <td style="vertical-align: top;">LB NINCDS</td> </tr> <tr> <td></td> <td style="vertical-align: top;">H. Walters</td> <td style="vertical-align: top;">Electronics Engr.</td> <td style="vertical-align: top;">LB NINCDS</td> </tr> </table>			PI:	W.J. Adelman, Jr.	Chief	LB NINCDS	Other:	K.S. Cole	Res. Biophysicist	LB NINCDS		R.J. French	Visiting Fellow	LB NINCDS		H. Walters	Electronics Engr.	LB NINCDS
PI:	W.J. Adelman, Jr.	Chief	LB NINCDS															
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	H. Walters	Electronics Engr.	LB NINCDS															
COOPERATING UNITS (if any): S. Takashima, Prof. Univ. of Pa., Phila, Pa.; C. Tyndale, Electronics Engr., MBL, Woods Hole, Mass.; Ri Waltz, Math. Programmer, MBL, Woods Hole, Mass.;																		
LAB/BRANCH <div style="text-align: center; font-weight: bold;">Laboratory of Biophysics</div>																		
SECTION <div style="text-align: center; font-weight: bold;">Section on Neural Membranes</div>																		
INSTITUTE AND LOCATION <div style="text-align: center; font-weight: bold;">IRP, NINCDS, NIH, MBL, Woods Hole, Massachusetts 02543</div>																		
TOTAL MANYEARS: <div style="text-align: center; font-weight: bold;">4.9</div>	PROFESSIONAL: <div style="text-align: center; font-weight: bold;">2.3</div>	OTHER: <div style="text-align: center; font-weight: bold;">2.6</div>																
SUMMARY OF WORK (200 words or less - underline keywords) <p> The general aim of this project has been to improve <u>electrical measurements</u> of <u>excitable membrane characteristics</u> consistent with physical and chemical methods for the study of nerve membrane ionic channels. Two major approaches were used. The first involved the development of methods for <u>rapid analysis</u> of ionic channel conductances by means of <u>computer controlled voltage clamp techniques</u>. A new data acquisition <u>system</u> for controlling voltage clamp experiments <u>on-line</u> and acquiring excitable membrane conductance data in a form that can be analyzed by digital computing machines has been designed and constructed. Programs for carrying out this analysis were developed. The second approach involved analysis of <u>excitable membrane characteristics</u> by means of <u>impedance measurements</u>. Improvement of <u>bridge impedance techniques</u> to achieve greater accuracy were made. <u>Impedance measurements on giant axons</u> and an investigation of effects of polarizations for comparison with step and ramp techniques and ion conduction models were carried out. This project was supportive of a number of other projects in terms of the development of relevant <u>hardware</u> and <u>software</u>. </p>																		

Project Description

Objectives:

- (1) Rapid analysis of ionic channel conductances by means of computer controlled voltage clamp: to develop modern approaches and systems for controlling voltage clamp experiments and acquiring excitable membrane conductance data in a form that can be analyzed by digital computing machines to develop programs for carrying out this analysis.
- (2) Steady-state analysis by means of impedance measurements: the improvement of bridge impedance techniques and accuracy on axon membranes and investigation of effects of polarizations for comparison with step and ramp techniques and ion conduction models.

Methods Employed:

- (1) The general method has involved the integration of general purpose data acquisition equipment into dedicated systems, suitable for controlling and acquiring data from biophysical and neurophysiological experiments.
- (2) An impedance bridge with internal guarded electrodes has been used and a new impedance bridge employing guarded external electrodes has been developed. This new "underground" bridge had been anticipated in 1969 by Robert Cole and coworkers at Brown University.

Major Findings:

- (1) A new computer controlled data acquisition system has been developed and put in operation at the Marine Biological Laboratory in Woods Hole. This system makes use of a PDP-11/10 digital computer, with dual floppy disks and 9-track read-write magnetic tape units as peripherals. One feature is a graphics terminal with hard-copy unit. The system is also interfaced via telephone line to large number crunching computers at DCRT in Bethesda and SIGMA 7 at Woods Hole Oceanographic Institution (WHOI) in Woods Hole. An operating program for voltage clamping axons has been designed and constructed. This system makes use of high speed 10 bit A/D and D/A converters. The software for program control has been developed and the system is now operational. The philosophy and design of this system has been used by the Section on Technical Development, NINCDS/NIMH as a model for construction of a number of other similar NIH systems.
- (2) Although "lossy dielectric" characteristics have defied theoretical explanation, they have been good descriptions of the properties of many cell membranes. But careful analysis of many data over many years have shown a number of curious, mostly small, examples of inconsistency and variability, several in clamp an impedance experiments on nerve and muscle. The Schwann layer so far effectively blocks the usefulness of high frequency data that have long been wanted. In some cases there have been indications or at least suspicions that the techniques allowed some fringing or end effects

at tissue or electrode boundaries. Two recent attempts to measure axon membrane impedance with internal guarded electrodes have been difficult in the already overcrowded axon interior - as well as controversial. However, an apparently novel circuit, called an "underground bridge", uses external measuring and guard electrodes. It functioned in preliminary tests but with unknown accuracy. Although its utility for the squid axon may be limited by internal electrode impedance and low signal to noise ratio at Woods Hole it should have several other applications, including elimination of edge effects particularly in thin tissues.

Proposed Course of Project: Continual development in this area is planned and expected.

Publications:

Adelman, W.J. Jr., and French, R.J. The Squid Giant Axon. Oceanus, Vol. 19, No. 2, pp. 6-16, 1976.

Cole, K.S. Neuromembranes: paths of ions. In Worden, F.G., Adelman, G., and Inogey, J.P. (Eds.): The Neurosciences: Paths of Discovery. Cambridge, MIT Press, pp. 143-157, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS 02087-03 LB
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Function and Structure of Ionic Channels: Ion Interactions and Gating Mechanisms		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	W.J. Adelman, Jr. R.J. French P. Mueller J. Wells	Chief Visiting Fellow Guest Worker Res. Physiologist
		LB NINCDS LB NINCDS LB NINCDS LB NINCDS
COOPERATING UNITS (if any): M. Cohen, Res. Assoc., Technion Med. School, Haifa, Israel; Y. Palti, Prof/Physiology, Technion Med. School, Haifa, Israel.		
LAB/BRANCH Laboratory of Biophysics		
SECTION Section on Neural Membranes		
INSTITUTE AND LOCATION IRP, NINCDS, NIH, MBL, Woods Hole, Massachusetts 02543		
TOTAL MANYEARS: 3.3	PROFESSIONAL: 3.3	OTHER: 0.0
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> Voltage clamp experiments were carried out to determine the function and structure of <u>ionic channels</u> in single giant nerve fibers. Information was obtained about the position and properties of membrane ionic channel sites that limit <u>conductances</u> and determine <u>ionic selectivity</u> by an analysis of the interaction between current-carrying and <u>blocking ions</u>. The ability of various <u>kinetic models</u> to describe the flow of ions through open <u>potassium channels</u> was tested. Kinetics of ionic blocking of channels were studied to gain information about <u>energy barriers</u> crossed by ions entering channels, and about the nature of <u>ion binding sites</u> within the channels. Analogies between the <u>kinetics of ionic currents</u> across the nerve membrane and those seen in excitable lipid bilayer membrane preparations treated with channel forming agents were investigated. Evidence was obtained to indicate that membrane <u>sodium current</u> is mediated by a <u>specific ion-site interaction</u>. </p>		

Project Description

Objectives;

(1) To obtain information about the position and properties of sites that limit membrane conductance and determine ionic selectivity, by analysis of interactions between current-carrying and blocking ions. To relate the dependence of the rate of the blocking reaction on temperature, voltage and ionic concentration to molecular events involved in the entry of ions into the channels. To test the ability of various kinetic models to describe ionic current flow through channels in which the gating mechanism is open.

(2) To investigate possible analogies between the kinetics of ionic currents across the axon membrane and those seen in lipid bilayer preparations treated with channel forming agents, and thus extend our understanding of time and voltage dependent gating of axonal currents.

(3) To investigate the possibility that membrane sodium current is mediated by a specific ion-site (SIS) interaction and that within the SIS framework the "ionic channel" mechanism may be treated using enzyme kinetics methods.

Methods Employed:

(1) Voltage clamp studies were performed on both internally perfused and intact squid giant axons bathed in modified artificial seawater containing 240 mM K^+ and various Cs^+ concentrations from 0 to 200 mM. TrisCl was added to keep ionic strength constant, and tetrodotoxin was added to the solutions so as to completely block sodium channels. Kinetics of Cs effects on both inward and outward currents were analyzed. Curve fitting and exploratory modelling was carried out using the MLAB program on DCRT's PDP-10 computer.

(2) Internally perfused squid giant axons were voltage clamped with appropriate pulse patterns so as to examine the kinetics of the opening and closing of both sodium and potassium channels. Observed channel relaxation kinetics were compared with the predictions of the Hodgkin-Huxley equations and the Baumann-Mueller molecular aggregation channel model.

(3) Squid giant axons were bathed in artificial seawater solutions in which both sodium concentration and potassium concentration were varied. Three potassium concentrations were used (10, 25, and 50 mM): for each potassium concentration, sodium was varied between 150 and 430 mM. The axons were voltage clamped in each of these solutions and sodium currents were recorded. The data was analyzed under the assumption that for a constant membrane potential (zero mV) the sodium conductance is determined by a specific ion-channel site (SIS) reaction.

Major Findings:

(1) The blocking reaction of external Cs with the K channel takes place over a time scale that is measurable with the voltage clamp technique (on the order of a msec) at low Cs concentrations. The electrical work done in moving a Cs ion onto the blocking site is greater than that which would be done moving a univalent ion through the whole membrane field. A physical picture consistent with the data is that there is a site capable of binding one Cs ion in the K channel, accessible from the external solution. In reaching that site a Cs ion displaces at least one ion through the membrane field and out of the channel. A previously unreported effect of external Cs, a small increase in the outward currents through the K channel, was described. The increase was dependent on Cs concentration, and was time dependent.

(2) The "inertia" seen previously in potassium channels following large brief depolarizations was confirmed and the study was extended. Extensive calculations were performed to examine predictions of the Hodgkin-Huxley model under similar circumstances. These calculations indicated a qualitative divergence between experimental results and Hodgkin-Huxley predictions. In contrast, the experimental observations were consistent with predictions of the Baumann-Mueller model. In another set of experiments, sodium channel relaxation kinetics were shown to be consistent with the Baumann-Mueller predictions and not Hodgkin-Huxley predictions. In addition to the above experiments and calculations, evidence was found for a potassium current inactivation mechanism.

(3) Sodium currents were shown to be proportional to sodium concentration and inversely proportional to potassium concentration. The sodium current density values were expressed in terms of SIS-reaction rates which were compared, by means of minimization techniques, with those computed for various reaction mechanisms. It was found that the dependence of peak inward sodium current on external sodium and potassium can be described in terms of saturation reactions. The experimental data fit well the kinetics of a positive cooperative homotropic reaction, involving at least two allosteric sites. One of these sites may be catalytic while the other, either catalytic or regulatory. The inhibitory effect of external potassium on inward sodium current, can be described by a reversible competitive or noncompetitive inhibition mechanism. The values of the dissociation constant of the inhibitor-site "complex" (K_i) were found to be close to the external potassium concentration under physiological conditions.

Proposed Course of the Project:

(1) Properties of the inner and outer mouths of the K channel are to be compared using Cs as a probe. Data for both external and internal Cs are already on hand. Studies of the properties of the inner mouth of the K channel are to be extended using Na and Li which are also known to block the channel when applied internally. Effects of temperature on the blocking reactions will be studied to obtain information about the energy barriers

that must be crossed by ions entering the channel. We hope to relate this data to the proposed dehydration step involved.

(2) Further calculations using both theoretical approaches (H-H & B-M) will be carried out. The experimental data and the results of these computations will then be prepared for publication.

(3) Plans are being made to examine other alkali cation (than K^+) interactions with sodium ions in sodium channels.

Publications:

Cohen, M., Palti, Y. and Adelman, Jr., W. J. Ionic dependence in sodium currents in squid axons analyzed in terms of specific ion "channel" interactions. J. Membrane Biol. 24: 201-223.

French, R.J. and Adelman, Jr., W.J. Competition, saturation and inhibition - Ionic interactions shown by membrane ionic currents in nerve, muscle, and bilayer systems. Current Topics in Membranes and Transport. (F. Bronner and A. Kleinzeller, eds.), Vol. 8, pp. 161-207, Academic Press, N. Y.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS 02092-03 LB
PERIOD COVERED July 1, 1975 to June 30, 1976			
TITLE OF PROJECT (80 characters or less) Subcellular and Extracellular Structures Associated with Nerve and Muscle.			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	W.J. Adelman, Jr.	Chief	LB NINCDS
	K.S. Cole	Res. Biophysicist	LB NINCDS
Other:	R.V. Rice	Guest Worker	LB NINCDS
	P. Roslansky	Guest Worker	LB NINCDS
COOPERATING UNITS (if any): D.O. Carpenter, AFRR; K.R. Foster, AFRR.			
R.V. Rice, Prof. of Biol., Carnegie Mellon Univ., Pittsburgh, Pa.			
P. Roslansky, Research Assoc., Carnegie Mellon Univ., Pittsburgh, Pa.			
LAB/BRANCH Laboratory of Biophysics			
SECTION Section on Neural Membranes			
INSTITUTE AND LOCATION IRP, NINCDS, NIH, MBL, Woods Hole, Massachusetts 02543			
TOTAL MANYEARS: 3.2	PROFESSIONAL: 3.2	OTHER: 0.0	
SUMMARY OF WORK (200 words or less - underline keywords)			
<p>The purpose of this project was to examine subcellular and extracellular structure of nerve and muscle fibers and relate such structure to function. Intermediate size neurofilaments from both squid and <u>Myxicola axoplasm</u> measure 10 nM in diameter. <u>10 nM filaments</u> found in <u>smooth muscle cells of chicken gizzard</u> apparently represent an underlying <u>cytoskeleton</u>. Presumptive evidence that both neurofilaments and muscle filaments contain the same protein (160,000 Daltons) was derived from <u>co-electrophoresis</u> experiments in which the protein isolated from chicken gizzard coincided with either squid <u>axoplasm protein</u> or with <u>Myxicola axon protein</u>. <u>Intermediate filaments</u> are found in many eucaryotic cells and are presumably important cellular components.</p> <p>On the basis of <u>electromicrographic reconstruction</u> of the <u>squid axon Schwann sheath structure</u>, a model was constructed which predicted a resistance in series with axolemma of 0.6 cm². Analysis of impedance measurements suggested that the impedance in series with the axon membrane can be represented by structural elements similar to those seen in the electromicroscope and that the <u>derived series resistance</u> has a value consistent with both anatomically and biophysically measurements. New values of <u>Loligo axon</u> and <u>Aplysia neuron cytoplasm</u> resistance confirmed previous estimates.</p>			

Project Description

Objectives:

- (1) To examine certain proteins in the cytoplasm of nerve and muscle fibers.
- (2) Both squid and *Myxicola* giant axons are enclosed in a sheath of Schwann cells (Geren and Schmitt, 1953) separated by roughly 100 Å wide clefts which extend from periaxonal space to outer basement membrane. Ion and water fluxes between axolemma and bulk extracellular fluids are assumed to move through a diffusion barrier formed by clefts between Schwann cells (Frankenhaeuser and Hodgkin, 1956; Villegas et al, 1962). Upon voltage clamping the giant axon, a resistance, R_s , in series with the axolemma is found. This must be compensated to measure membrane conductances accurately (Hodgkin et al, 1952). Current clamp (Binstock et al, 1975) and voltage clamp (Frankenhaeuser and Hodgkin, 1956) methods are used to measure R_s . The objective of this part of the project was to obtain an anatomical Schwann sheath model for the series resistance and to correlate predictions of this model for the value of the series resistance with values obtained by means of biophysical and physiological experimentation.
- (3) To determine the resistance in series with the excitable membranes of giant axons.
- (4) To determine the resistivity of neuronal cytoplasm.

Methods Employed:

(1) Giant axons from the squid, *Loligo pealei* were carefully dissected, cleaned of connective tissue and blotted. The axoplasm was carefully rolled out so that no external material contaminated the axoplasm. Axoplasm was stored at -70°C until ready for use. Polyacrylamide gel electrophoresis was carried out according to the method of Fairbanks et al. (Biochem. 10:2606 (1971)) using 5.6% gels containing 1% sodium dodecyl sulfate (SDS). The gels were calibrated with proteins of known molecular weight according to the method of Weber and Osborn (Biochem. 244:4406 (1969)) using paramyosin, albumin, ovalbumin, chymotrypsin, myoglobin, and gamma globulin as standards. Squid axoplasm was prepared for electrophoresis in two ways. Whole axoplasm was ground in 10 mM Tris pH 7.2, 1 mM EDTA, 1% SDS and applied directly to the gel, or more usually ground and dialyzed against this solution overnight. Some purification and separation from large amounts of tubulin and smaller molecular weight proteins was achieved by grinding in an ice-cold solution containing 0.6 M KCl, 5 mM MgCl₂, 10 mM Tris pH 7.2 and 40 mM Clelands reagent and then centrifuging at 50,000 RPM for three hours. The precipitate was dissolved in Tris-SDS-EDTA and then dialyzed overnight. All proteins were treated with 1% SDS and 40 mM Clelands reagent and boiled 2 minutes before electrophoresis.

The axon from the sabellid worm Myxicola infundibulis was dissected from the dorsal side. Two cuts the length of the worm were made on either side of the fecal groove. The skin was peeled off between these two cuts and the axon lay beneath only a thin layer of muscle and connective tissue. The axon was lifted out and cleaned as carefully as possible and stored at -70°C until used. Myxicola axons were best prepared for electrophoresis by grinding and dissolving in an ice-cold solution containing 0.6 M KCl, 5 mM MgCl_2 and 10 mM Tris pH 7.2. This suspension was left cold overnight and ATP (0.015 gms 10 ml) was added for 1 hour in the morning. This suspension was centrifuged 15 minutes at 20,000 RPM and the clear supernatant was used for electrophoresis - after dialysis to Tris -SDS-EDTA. Proteins can be isolated from the various bands by cutting out the unstained band and eluting 24-48 hours in 10 mM Tris pH 7.2 and 1% SDS. Protein concentrations were determined by the method of Lowry (Biochem. 193:265-272 (1951)). Preparations were negatively stained using 2% uranyl acetate and examined in a JEOL transmission electronmicroscope.

(2) To correlate R_s with sheath anatomy, axons of Loligo pealei were fixed and stained. Cross and longitudinal sections were examined by transmission electronmicroscopy. Collage photographs were assembled and measurements made of width and frequency of clefts entering the sheath from the periaxonal space and leaving the sheath at the basement membrane.

(3) The original 1947 method to estimate R_s as well obtain the most direct value for membrane capacity was to apply a step of constant current from an axial electrode to a guarded external electrode and measure the transient potential between them. This is still the simplest and most direct in concept. However, as equipment and experiments developed in power and speed the current step has not been made instantaneous on the μ sec scale and a correction is needed. This is relatively simple for an approximately exponential current rise but of uncertain accuracy otherwise-- such as for the slow initial rise which is sometimes hard to avoid.

(4) The several methods for measurement of cytoplasmic resistivity without cell invasion are indirect and have given inconsistent and widely varying results in many forms which seem not to have been explained in any single case. Recent investigations of axons and ganglia within a single small internal electrode have resulted in even wider spread of data and some uncertainty of the contributions of the electrode impedances. Although there was a wide spread of values for squid axoplasm, they were in the range of some earlier conclusions. Another approach was to measure the resistance between two small electrodes as they were moved toward each other from the ends of an axon immersed in sucrose. Ion loss across the membrane made this impractical so the same procedure was used on axoplasm extruded into a glass capillary.

Major Findings:

(1) Preparations of squid and Myxicola axoplasm were examined both in the electronmicroscope using negative staining techniques and by SDS gel electrophoresis. Electronmicroscopy demonstrated the presence of filaments 100 angstroms in diameter in both Myxicola and squid preparations.

Gel electrophoresis of squid axoplasm using either whole ground axoplasm or the centrifuged solubilized pellet separated twelve or more different proteins in a reproducible pattern. The same bands were obtained in seven different squid preparations. Major proteins have the following molecular weights: 250,000; 200,000; 150-170,000; 130,000; 100,000; 82,000; 62,000; 57,000; 45,000; 41,000; 22,000 daltons.

Smooth muscle preparations from chicken gizzard and cow stomach studied by R. V. Rice at Carnegie-Mellon Univ. contained filaments 100 angstroms in diameter. These represent an intermediate sized filament which is larger than the thin filaments (actin 60-80A) and smaller than the thick filaments (myosin 150A). These intermediate sized filaments appear to be associated with a protein which has a molecular weight of around 160,000 daltons as determined by SDS gel electrophoresis. This protein has been isolated by slicing and eluting the unstained bands obtained on the gel. When the purified protein was co-electrophoresed with centrifuged squid preparations, the bands coincided.

Gel electrophoresis of Myxicola axons demonstrated the presence of many proteins, some of which were not derived from axoplasm but from the surrounding muscle and connective tissue. Major bands show proteins of the following molecular weights: 215,000; 178,000; 102,000; 42,000 daltons. This preparation was co-electrophoresed with the protein isolated from chicken gizzard and the band at 178,000 coincided with the chicken gizzard protein.

Preparations were also made from the anterior byssus retractor muscle of Mytilus edulis. These preparations contain 100 angstrom filaments but the gel electrophoresis has not been reproducible.

(2) The average cleft width = 108 Å. One cm² of axon surface has a sheath cleft area of 0.001 cm² at the periaxonal surface and 0.017 cm² at the basement membrane, the clefts branching frequently as they cross the sheath. A model was used to predict R_s . Assuming the clefts contain seawater and can be lumped into a truncated cone with the narrower radius at the inner surface, then (normalized for 1 cm² of axon surface) $R_s = (SL)/(\pi r_1 r_2)$, where S = specific resistance (25 Ω cm), L = width of Schwann cell layer (1 μ), r_1 = inner diameter of the cone (0.0184 cm) and r_2 = outer diameter (0.0727 cm). Thus, $R_s = 0.6 \Omega$ cm². This value compares favorably with the current clamp value of 0.85 Ω cm² and the voltage clamp value of 1.37 Ω cm² (Adelman *et al.*, 1973), as these electrically measured R_s values include the resistance of the outer connective tissues.

(3) A paper has been published of an analysis of a dispersion in the first axon data of 1937 which gives a reasonable value for R_s and a capacity, open to interpretation, for the Schwann sheath. A note has been published generalizing the current method for measuring R_s . The analogue solutions for the Laplace equation showed the dilute solution to hold, without analysis, for such a dense tissue. A similar analogue solution for square, membrane covered cylinders, appearing in December, similarly followed the dilute analysis for the capacity - but application to the complex sheath structure is uncertain. It seems highly probable that the analogue solutions for dense three dimensional tissues will similarly extend the dilute results. These should be made, but even now the results pose a challenge to the powers of mathematical Physics which have done so much with the Laplace equation.

(4) A paper on squid axoplasm resistivity was published with another by the AFRRRI Group and they mostly confirm the best of previous values. The Aplysia work on cytoplasmic resistivity has been extended to higher frequencies with better indications of an axoplasmic conductance comparable to squid. A lack of evidence for membranes and the high apparent electrode impedances in the ganglion are unexplained - unless they can be shown to be cause and effect. This work will be discontinued at AFRRRI, but might be continued at the University of Pennsylvania soon.

Proposed Course of Project:

(1) We will prepare milligram quantities of the protein (160,000D) from squid axoplasm and myxicola. These proteins will be compared with that isolated from chicken gizzard at Carnegie Mellon University using cyanogen bromide peptide maps and analyses of their amino acid compositions.

(2) The anatomical modeling of the Schwann sheath is being extended to the sheath of Myxicola axons.

(3) The Schwann sheath on squid axons will not be continued except to look for a rational analysis of irregular membrane geometrics and hope for comparisons of the extracellular volumes from impedance and electron-micrograph data.

More analysis is needed to account for the 5 - 10% discrepancies between calculated and measured membrane capacities for square cylindrical cells in square array. Analysis and analogs have been given for seven cases. Analog experiments on the capacity of membrane covered cubes in cubic array have been started. If the several problems are solved and the answer is as expected, the value of impedance measurements will be considerably increased in general and the mathematical challenges to explain the high concentration Laplace solutions will be yet stronger.

Publications:

- Adelman, W.J. Jr. Modification of axonal conductance and excitability in squid axons by periaxonal potassium: Implications for information processing in neural systems. Soc. for Neurosci., 5th Annual Meeting. Summaries of Symposia (BIS Conference Report #43). UCLA, Los Angeles, Brain Information Service/BRI Publications Office, 1976. pp. 84-93.
- Cole, K.S. Resistivity of Axoplasm. I. Resistivity of Extruded Squid Axoplasm. Journ. Gen. Physiol. Vol. 66: 1330138, 1975.
- Cole, K.S. Analogue Solution for Electrical Capacity of Membrane Covered Square Cylinders in Square Array at High Concentration. Proc. Nat'l. Acad. Sci. USA, Vol. 72(12): 4936-4939, 1975.
- Cole, K.S. and Lecar, H. On the Measurement of Series Resistance in Giant Axon Preparations. Journ. of Membrane Biol. Vol 25: 209-212, 1976.
- Cole, K.S. Electrical Properties of the Squid Axon Sheath. Biophys. J., Vol. 16: 137-142, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS 02151-02 LB
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Information Processing in Simple Nervous Systems		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	D.L. Alkon T. Akaike E. Heldman T. Crow	Medical Officer Visiting Associate Staff Fellow Extramural Fellow LB NINCDS LB NINCDS LB NINCDS LB NINCDS
COOPERATING UNITS (if any) J. Harrigan, Res. Assoc., Marine Biological Laboratory, Woods Hole, MA 02543		
LAB/BRANCH Laboratory of Biophysics		
SECTION Section on Neural Systems		
INSTITUTE AND LOCATION IRP, NINCDS, NIH, MBL, Woods Hole, MA 02543		
TOTAL MANYEARS: 6.0	PROFESSIONAL: 6.0	OTHER: 0.0
SUMMARY OF WORK (200 words or less - underline keywords) <p> The principal objective of this project is to study the mechanisms by which simple <u>neural networks</u> process information with particular emphasis on <u>mechanisms</u> of learning. The nervous system of <u>Hermisenda crassicornis</u> has proven to be an excellent model for <u>information processing</u> at several levels: <u>sensory transduction</u> by photoreceptors and hair cells, analysis of <u>synaptic circuitry</u>, changes in synaptic circuitry produced by conditioning <u>paradigms</u> administered to intact animals as well as to isolated nervous systems, membrane properties modified by <u>conditioning</u>, identification of critical developmental stages for the neural networks studied as well as stages critical for learning. </p> <p> Techniques employed thus far to pursue these questions include: <u>simultaneous intracellular recording</u> from multiple neural elements, paired stimulation of the visual and vestibular pathways using a rotating table, iontophoresis of fluorescent dyes and electron dense materials, automated behavioral monitoring of intact <u>Hermisenda</u>. Other methods include biochemical and developmental approaches to the above problems. These include <u>mariculture</u>, <u>subcellular fractionation</u> protein phosphorylation analysis, and uptake of <u>neurotransmitter precursors</u>. </p>		

Project Description:Objectives:

(1) To study the mechanisms by which simple neural networks process information with particular emphasis on mechanisms of learning. Information processing at several levels is of interest:

- a. Sensory transduction by photoreceptors and hair cells.
- b. Synaptic interactions between primary sensory receptors.
- c. Synaptic interactions between primary and higher order neural elements.
- d. Intersensory communication: e.g. synaptic interaction between the visual and gravitational sensory pathways.
- e. Changes of synaptic interaction produced by conditioning paradigms administered to the intact animals as well as to the isolated nervous systems.
- f. Membrane and synaptic properties modified by conditioning.
- g. Identification of critical developmental stages of the neural networks studied as well as stages critical for learning.

Methods Employed:

(1) The nudibranch mollusc Hermissenda crassicornis is the principal experimental preparation. Other marine species are also being screened to provide favorable preparations for specific experimental questions. Intracellular recording from several neural cells simultaneously has been the main technique used thus far. Means for simultaneously stimulating the visual and vestibular pathways (which has permitted conditioning) while recording intracellular potentials have been developed in our laboratory. Iontophoresis of fluorescent dyes (e.g. Procion Yellow) and electron dense materials (e.g. cobalt) are also being used extensively.

(2) Other methods allow biochemical and developmental approaches to the problems of interest. These include mariculture, subcellular fractionation, protein phosphorylation analysis, uptake of neurotransmitter precursors, etc. Automated behavioral monitoring now permits long-term studies of intact Hermissenda.

Major Findings:

(1) Past work has focused on four major areas:

- a. Receptor physiology,
- b. Neural network analysis,
- c. Behavioral conditioning with neural correlates, and
- d. Cellular conditioning in isolated nervous systems.

(2) Hair Cell Physiology: With an improved rotatory stimulus it has been possible to characterize more exactly the nature of adequate stimulation of hair cells. Specifically, hair responses to changes in angular

acceleration could be carefully compared to responses to changes in angular velocity. Stimulus threshold, the role of adaptation, stimulus-response relationships, and hair cell noise have also been carefully investigated.

(3) Cellular Conditioning: Stimulus paradigms have been developed for stimulation of the visual and vestibular pathways in isolated Hermisenda nervous systems. These paradigms can now be characterized as conditioning paradigms because of their required temporal specificity, their cellular specificity, and the long-lasting neural changes they produce. Type A but not Type B photoreceptors respond with fewer impulses to a light stimulus following repeated temporally specific pairing of light and rotation. This conditioning effect can now be explained by a mechanism involving a long-term network bias: Type B photoreceptor inhibition of Type A cells is enhanced by pairing the inhibitory effect (through the hair cells) of rotation with the exciting effect of light. This network bias was further demonstrated by simultaneously recording from the pre- and post-synaptic elements, the Type B and A photoreceptors.

(4) Regulation of Impulse Activity by Extracellular Potassium: The role of changes in extracellular potassium for regulation of impulse activity has been examined for Type A and B receptors. Type A impulses are much more sensitive than Type B to changes in extracellular potassium. This could be explained largely by inactivation due to membrane potential elevation. Significant accumulation of potassium occurred following Type A light responses.

These phenomena appear to bias the photoreceptor network in each eye against Type A firing and for Type B activity. Such a bias might pre-dispose the network to the longer term changes observed in the conditioning studies.

(5) Electronmicroscopic Identification of Synaptic Junctions: Techniques for iontophoretic injection of the electron dense substances, cobalt and horseradish peroxidase, have been successfully adapted for identifying synaptic junctions in Hermisenda. Histologic processing, involving both histochemical and cryostatic methods, has also been developed. These techniques, in conjunction with past and ongoing electrophysiologic studies, should provide conclusive demonstration of monosynaptic connections within the neural networks of Hermisenda and possibly other relatively simple marine forms of interest.

(6) Biochemical Analyses: Neurotransmitter substances have been identified and characterized in the isolated circumesophageal nervous system as well as isolated structures such as the eyes, statocyst, and optic ganglia of Hermisenda. Uptake of radioactive precursors and synthesis of neurotransmitter substance, particularly acetylcholine, have been observed in these structures. Single cell analyses are now being prepared. These

will be performed in conjunction with other experiments involving iontophoretic injection of radioactive precursors and metabolites in single cells.

(7) Behavioral Experiments: An automated apparatus for long-term behavioral monitoring has been assembled. This will permit testing of conditioning paradigms on intact Hermisenda over many weeks and possibly months. The proper natural environment for continued maintenance of animals to be tested in these experiments can now be provided utilizing the seaside holding facilities of the Marine Biological Laboratory.

(8) Mariculture: Hermisenda larvae have been successfully raised to the final premetamorphosis stage. Triggering substances are now being screened for metamorphosis induction. Nutrients have also been screened for adult animals and Hermisenda shipped from the Pacific grow considerably with the feeding and maintenance procedures developed in the last several months.

Proposed Course of Project:

(1) Precise analysis of synaptic interactions between cells within the aforementioned neural networks will be continued using the techniques of intracellular recording and iontophoresis. Particular emphasis will be placed on electronmicroscopic visualization of cell contacts aided by axonal absorption of hydrogen peroxidase and/or cobalt. These studies will not be limited, however, to the networks already discussed. A third sensory pathway, that mediating chemoreception, will also be mapped. In addition, other marine forms with potentially analyzable neural networks and behavior will be explored.

(2) Detailed noise analyses will be conducted on hair cell potentials with and without physiologic stimulation. Efforts will be made to identify the behavior of membrane ionic channels responsible for stimulus transduction in the hair cells.

(3) Cellular mechanisms responsible for the learning identified in the intact animal and its isolated nervous system will be further analyzed. Careful attention will be given to synaptic processes responsible for the long-term inhibition believed to underly cellular conditioning.

(4) Biochemical and pharmacologic analyses of neurotransmitter substances within the visual and vestibular pathways will be continued. Studies are also planned to identify subcellular and/or biochemical loci for the neural changes produced by the conditioning paradigms used.

(5) Behavioral experiments will be performed to ascertain the comparability of cellular conditioning and behavioral conditioning as well as the comparability of changes observed for Hermisenda to changes observed for animals with more evolved nervous systems.

(6) Hermisenda will be raised through successive generations. It is planned to combine all of the above approaches with mariculture. The existence of clearly identified neural networks in Hermisenda together with mariculture offer for the first time the possibility of using genetic mapping to study principles of neural organization, development, and learning.

(7) The many marine forms available at the Marine Biological Laboratory (MBL) will be screened to identify other neural networks and synaptic junctions. The generalizability of principles and phenomena observed in Hermisenda may then be tested and compared for other evolutionary stages.

Publications:

Alkon, D.L. Responses of Hair Cells to Statocyst Rotation. The Journal of General Physiology, Vol. 66: 507-530, 1975.

Alkon, D.L. Signal Transformation with Pairing of Sensory Stimuli. Journal of General Physiology, Vol. 67: 197-211, 1976

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Function and Structure of Membrane Ionic Channels: Pharmacology and Ionic Selectivity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Binstock	Electronic Engineer	LB NINCDS
Other:	R.E. Taylor	Supv. Res. Physiologist	LB NINCDS
	L.M. Huang	Guest Worker	LB NINCDS
	W.A. Catterall	Staff Fellow	LBG NHLI
	E. Ehrenstein	Research Physicist	LB NINCDS

COOPERATING UNITS (if any): C. M. Armstrong, Prof., Univ. of Penn.; F. Bezanilla, Prof. Univ. of Chile.

LAB/BRANCH

Laboratory of Biophysics

SECTION

Section on Molecular Biophysics

INSTITUTE AND LOCATION

IRP, NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

3.9

PROFESSIONAL:

2.0

OTHER:

1.9

SUMMARY OF WORK (200 words or less - underline keywords)

The long-range purpose of this project is to study the function and structure of membrane ionic channels. The areas of present research are:

- 1) The permeability of organic cations in the sodium channel of Myxicola,
- 2) The influence of ionic strength on ionic selectivity.
- 3) The effects of neurotoxins on the axonal membrane in Myxicola;
- 4) The kinetics of various ions (mainly calcium, magnesium and cesium) through the sodium channels of the squid giant axon.
- 5) The influence of charge on ionic permeability in tissue-cultured cells derived from chick embryo muscles.

Project Description

Objectives: To study the ionic current flow across the nerve membrane without the complication of excitation and propagation in terms of individual ion currents and to observe the effect of changes in normal external and internal environments. These environments are altered by addition of various chemical and pharmacological agents as well as by changing the ionic environment. Also to study the effect of charge of the transporting molecules on the rate of their permeation through acetylcholine channels. The long range objectives are the interpretations of the structures and mechanisms by which the permeabilities are controlled.

Methods Employed: Standard voltage clamp techniques are employed on preparations a and b: (a) Myxicola giant axon. For many experiments on the giant axon of Myxicola, the voltage clamp is under computer control; (b) Squid giant axon. The squid giant axon is internally perfused and signal averaging techniques are used. Standard radioactive counting techniques are employed with preparation (c): (c) Cultured muscle cells from chick embryo. Carbamylcholine is used to open the acetylcholine channels in these cells, and radioactive traces are used to measure ionic uptake.

Major Findings: (a) Myxicola giant axon: The relative permeability of sodium channels to organic cations was determined. Ionic currents under potential control were measured in sea water and in sodium-free solutions containing the organic cation. The measured reversal potential and the Goldman equation were used to obtain the relative permeabilities. The permeability sequence was found to be: sodium > hydrazine > ammonium > guanidine > formamidine > aminoguanidine. Measurements were also made on sodium and several of the organic cations at different concentrations. The relative permeabilities of the cations were found to be independent of concentration. A manuscript has been completed and is being prepared for publication.

The neurotoxin, veratridine, produces a decrease in sodium and potassium currents and a decrease in the sodium equilibrium potential. The leakage current is increased.

(b) Squid giant axon: It was found that external calcium ions interfere with the movement of sodium ions through the sodium channels. The data were fitted by a model which assumes that a calcium ion temporarily occludes the channel when it occupies a site about halfway through the membrane field.

(c) Cultured muscle cell: The acetylcholine channels are substantially blocked as the external pH is lowered. The dependence of sodium permeability versus pH is a simple titration curve with pK around 4.6.

The charges on the molecules affect the rate of their permeation. The molecules tested so far, included tris (hydroxymethyl) aminomethane and methylamine. The charged form of both these molecules are more permeable than their uncharged form.

Significance to Biomedical Research and the Program of the Institute: The determination of the relative permeabilities of different ions through sodium channels of Myxicola provides information to test hypotheses that the sodium channel is lined with oxygen atoms. Furthermore, the results strongly suggest that widely differing species have sodium channels that are very similar.

The result that calcium ions can bind at a particular site in the sodium channel not only provides information about the channel, but also offers a possible method for blocking sodium channels.

The preliminary result that charge plays a crucial role in determining ionic permeability is also of considerable value in determining the mechanism for cationic selectivity in channels. In particular, this result shows that the cation selectivity does not depend primarily on cation size (cations tend to be significantly smaller than anions), but on the electrical charge, itself.

Proposed Course of Project: Study mixtures of organic cations and sodium for competition. Study the following toxins in greater detail: veratridine, batrachotoxin, aconitine, and scorpion venom. It has been reported (Catterall, PRAS, 72: 1782, 1975) that interactions of these toxins have been examined by means of ^{22}Na on excitable cell cultures where specific sites have been found. Myxicola axons will be voltage clamped to further study the interaction of these toxins in order to:

- (1) Observe the ionic currents, (2) Study the kinetics to see if there are any changes in the membrane, (3) Determine if the neurotoxin sites are related to the gating mechanism, by measuring gating currents before and after application of the toxins. Additional experiments on the squid giant axon are planned to further quantify the results. In particular, it is proposed to attempt to measure the instantaneous current voltage curve for the calcium current through the sodium channels. For acetylcholine channels in cultured muscle cells it is planned (1) to study the permeability of several other organic molecules in order to confirm present findings; (2) to measure potassium permeability with increase in proton concentration and to check whether there is much difference between sodium and potassium permeability as a function of pH in the Ach channels; and (3) to investigate the effect of pH and charge on the cation permeability through sodium channels.

Publications:

Keynes, R.D., F.R.S., Bezanilla, F., Rojas, E. and Taylor, R.E.: The

rate of action of tetrodotoxin on sodium conductance in the squid giant axon. Phil Trans. R. Soc. Lond. B. 270: 365-375, 1975.

Rojas, E. and Taylor, R.E.: Simultaneous measurements of magnesium, calcium and sodium influxes in perfused squid giant axons under membrane potential control. J. Physiol. 252: 1-27, 1975.

WILSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 NS 02089-03 LB</div>
PERIOD COVERED <div style="text-align: center;">July 1, 1975 to June 30, 1976</div>		
TITLE OF PROJECT (90 characters or less) <div style="text-align: center;">Electrical Fluctuations in Excitable Cells</div>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: H. Lecar Other: G. Ehrenstein F. Sachs	Research Physicist Research Physicist Staff Fellow	LB NINCDS LB NINCDS LB NINCDS, SUNY, Buffalo
COOPERATING UNITS (if any) <div style="text-align: center;">Physiological Laboratory, Cambridge, England</div>		
LAB/BRANCH <div style="text-align: center;">Laboratory of Biophysics</div>		
SECTION <div style="text-align: center;">Section on Molecular Biophysics</div>		
INSTITUTE AND LOCATION <div style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</div>		
TOTAL MANYEARS: <div style="text-align: center; font-weight: bold;">1.0</div>	PROFESSIONAL: <div style="text-align: center; font-weight: bold;">0.8</div>	OTHER: <div style="text-align: center; font-weight: bold;">0.2</div>
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> <u>Electrical Fluctuations</u> were measured in <u>tissue-cultured skeletal muscle cells</u> in order to determine the unit conductance of <u>excitable ionic channels</u>. Theoretical studies were performed in an attempt to reconcile the existing data on <u>gating currents</u>, conductance-activation kinetics (<u>voltage-clamp</u> data) and electrical <u>noise</u> fluctuations as complementary ways to explore excitability. </p>		

Project Description

Objectives: To determine the conductances and other transport properties of ionic channels in excitable membranes. To develop a molecular model of the gating process by which the channels regulate ion permeability during excitation.

Methods Employed: Electrical fluctuations are measured by monitoring the changes in electrical current noise induced when neurotransmitters are iontophoretically released at the postsynaptic membrane. Chick skeletal muscle cells grown in tissue culture are altered by vinblastine and colchicine application and electrical measurements are made using a two-microelectrode voltage clamp. Current noise spectra are recorded by on line computation using a Fast-Fourier Transform program.

Theoretical analysis of the gating-current phenomena and of the spectrum of excitability fluctuations was performed in conjunction with an analysis of the kinetics of the conductance transients in electrically and chemically excitable membranes.

Major Findings: The experiments on acetylcholine and carbachol noise in tissue cultured muscle cells have been completed and a manuscript has been prepared for publication. The major results are the determination of a value of 39 pS for the conductance of the post-synaptic channel, at a temperature of 25°C. The conductance increases with temperature, with a Q_{10} of 1.6, as might be expected for a low-barrier hydrated pore. From the postsynaptic power spectrum, the relaxation time of the gating process was determined, and shown to have a sharp temperature dependence, $Q_{10} = 5$. Carbachol experiments showed a similar conductance per channel, but a faster conductance relaxation time.

Significance to Biomedical Research and the Program of the Institute: The study of chemical transmission at the neuromuscular synapse by electrical fluctuation methods provides a means of understanding the nature of chemically mediated synaptic transmission, which is the most important membrane process underlying integration in the central nervous system.

The study of the effects of vinblastine treatment on tissue-cultured excitable cells has established the feasibility of using these cells as a stable preparation for voltage-clamp analysis. This method can lead to the extension of noise analysis to other varieties of excitable cells in tissue culture.

Proposed Course of Project: The noise studies of Ach channels in tissue-cultured muscle cells have been completed. The microelectrode voltage clamp and electrical noise measurements will now be modified for the study of other types of excitable cells. The theoretical analysis of gating transients, noise spectra and conductance kinetics will be continued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS 02090-03 LB
PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p>		
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Lipid Bilayer Membranes</p>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	G. Ehrenstein H. Lecar R. Holz B. Pailthorpe F. Sachs	Research Physicist Research Physicist Guest Worker Visiting Fellow Asst. Professor
		LB, NINCDS LB, NINCDS BB, NICHD LB, NINCDS LB, NINCDS
COOPERATING UNITS (if any): R. Latorre, Asst. Prof., Univ. of Chicago; O. Alvarez, Asst. Prof., Univ. of Chile; M. Espinoza, Instructor, Univ. of Chile; J. Reyes, Instructor, Univ. of Chile.		
LAB/BRANCH <p style="text-align: center;">Laboratory of Biophysics</p>		
SECTION <p style="text-align: center;">Section on Molecular Biophysics</p>		
INSTITUTE AND LOCATION <p style="text-align: center;">IRP, NINCDS, NIH, Bethesda, Maryland 20014</p>		
TOTAL MANYEARS: <p style="text-align: center;">1.8</p>	PROFESSIONAL: <p style="text-align: center;">1.5</p>	OTHER: <p style="text-align: center;">0.3</p>
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> The aim of the project is to incorporate <u>ionic channels</u> in <u>lipid bilayers</u> and to study their properties - particularly their electrical properties. In particular, we have studied <u>hemocyanin</u> channels and <u>EIM</u> (excitability-inducing material of bacterial origin) channels. Both of these channels exhibit <u>negative resistance</u> with moderate doping concentrations. One long-range objective is to relate this negative-resistance behavior to properties of single channels. We have recently started another program, whose purpose is to incorporate biological channels into lipid bilayers, primarily by <u>membrane fusion</u>. The long-range objectives of this program are to determine <u>single-channel</u> properties of biological channels as well as to learn more about <u>membrane fusion</u>. </p>		

Project Description:

The study of several different voltage-dependent ionic channels allows comparisons of the channel properties and their gating mechanisms. EIM and hemocyanin (and other bilayer channels investigated elsewhere) all seem to have discrete conductance levels with conductance differences of the order of 100 pS. The number of discrete levels, on the other hand, shows considerable variability. EIM has only two conductance levels, and thus represents the simplest system that can have negative resistance. We have recently shown that hemocyanin has at least five discrete conductance levels and a large number of sublevels within each of the main levels. Transitions between sublevels are at least 1000 times faster than transitions between main levels.

It is likely that each conductance state represents a particular molecular conformation. Thus, different channels have different numbers of stable conformations and different voltage-dependent transition rates between the conformations. These are differences in details. All of these channels change their conductance in response to membrane voltage, and the responses are sufficiently voltage-dependent to produce negative resistance when the membrane contains many channels.

We hope to extend these results to the determination of single-channel properties of biological channels. The most difficult step in this direction is the incorporation of these channels in a bilayer. We are trying to obtain channel incorporation by fusion of small cells with lipid bilayers. In attempting to do this, we plan to try several different lipids and several different cells. We also plan to search for experimental conditions (such as ionic strength and temperature) that will be conducive to membrane fusion.

Spin-label ESR experiments provide a means of assessing changes in lipid structure. An experiment performed with this technique demonstrated that the interaction of human platelets with thrombin does not involve any substantial change in lipid organization. Another experiment using this technique is planned to test whether local anesthetics affect the lipid organization of nerve membranes.

Publications:

Alvarez, O., Diaz, E., and Latorre, R.: Voltage-Dependent Conductance Induced by Hemocyanin in Black Lipid Films. Biochimica et Biophysica Acta 389: 444-448 (1975).

Latorre, R., Alvarez, O., Ehrenstein, G., Espinoza, M. and Reyes, J.: The Nature of the Voltage-Dependent Conductance of the Hemocyanin Channel. J. Memb. Biol. 25: 163-182 (1975).

Z01 NS 02090-03 LB

Lecar, H., Ehrenstein, G., and Latorre, R.: Mechanism for Channel Gating in Excitable Bilayers. NY Acad. Sci. Vol 264: 304-313 (1975).

Sachs F. and Feinman, R.D.: Spin-Labelled Human Platelets. Thrombosis Res. 8: 43-50 (1976).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02091-03 LB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Mathematical Modeling

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. FitzHugh	Research Physicist	LB NINCDS
OTHER:	J. Rinzel	Research Mathematician	MRB NIAHDD
	G. Ehrenstein	Research Physicist	LB NINCDS

COOPERATING UNITS (if any)

Mathematical Research Branch NIAHDD

LAB/BRANCH

Laboratory of Biophysics

SECTION

Section on Molecular Biophysics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

1.3

PROFESSIONAL:

1.1

OTHER:

0.2

SUMMARY OF WORK (200 words or less - underline keywords)

Mathematical models for the following phenomena were studied.

- 1) The dependence of threshold to anodal break excitation on temperature in the Hodgkin-Huxley nerve equations.
- 2) Optimal motor control functions required to minimize either total energy expended or total time of a muscular motion.
- 3) The mathematical basis of periodic bursts of impulses in a simple model of a bursting pacemaker.
- 4) The development of interactive computer programs for solving nerve membrane equations in current and voltage clamp and for threshold search.

Project Description:

Computations of anodal break excitation in the Hodgkin-Huxley equations showed the unexpected appearance of a critical temperature, above which anodal break excitation cannot be elicited. Mathematical analysis of the equations showed that this phenomenon originates in the saturation of the conductance variables (m , h , n) for strongly hyperpolarized membrane potentials. Since such saturation is a general property of membrane model the search for this critical temperature by improved experimental methods would help to test present and future models. This portion of the project has been completed.

The optimal motor control input signal to a simple muscle model without series elastic element, acting against a combined constant and inertial load, previously determined for minimum total energy, has now been found also for minimum total time. For a lengthening muscle, and for shortening with a large enough mass and/or a small enough shortening distance, the two solutions are identical, but can differ appreciably for smaller masses and larger distances. Such analysis by optimal control theory provides a testable hypothesis for predicting muscular motor inputs, which could be extended to more complicated and realistic models including more than one muscle, and limb constraints. Further extensions of the method include models with series elastic element, and dependence of tension on muscle length.

In collaborating with Dr. John Rinzel, a simple model of a bursting pacemaker, formed by adding a new equation to the BVP model of the nerve membrane, has been studied in an attempt to discover how it produces regular and irregular sequences of impulse bursts. These bursts have been observed in both analog and digital computations with the model. They appear to be the result of the slow oscillatory interaction of the new slow variable, which mimics a stimulating current, with the averaged membrane potential. This causes the alternate appearance and disappearance of the faster oscillation representing the repetitive firing of impulses. The reason for the irregularity is not yet clear. It is hoped that nonlinear mathematical analysis will explain the observed properties of bursting pacemaker neurons, in terms of more accurate models based on experiments.

The interactive computer programs which have been developed for the convenient solution of the Hodgkin-Huxley and other membrane equations under current clamp, including threshold searches, have been made available to the public through NTIS. Other programs for solving modified Hodgkin-Huxley equations under voltage clamp have been developed for the use of laboratory members in Woods Hole. Future developments may include the modification of the earlier programs to include ramp input currents.

An analysis of spherical aberrations in lenses has been made for the purpose of clarifying the reason for the characteristic dependence of of aberrations on the third power of the distance from the axis. It was found that this dependence is based on general relations between the radial and axial terms in Laplace's equation.

Publications:

FitzHugh, R.: Nerve Membrane Model. FORTRAN programs to compute solutions to Hodgkin-Huxley equations. Magnetic tape distributed by National Technical Information Service, U. S. Dept. of Commerce. NTIS Accession No. PB243847/AS. 1975.

FitzHugh, R.: Anodal excitation in the Hodgkin-Huxley nerve model. Biophys. J. 16: 209-226. 1976.

Ehrenstein, G.: On the derivation of spherical aberrations. Amer. J. Physics 43: 745. 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS 02218-01 LB
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Voltage-Dependent Ionic Conductance in Membranes		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	D.L. Gilbert G. Ehrenstein Li-Yen Huang F. Sachs	Physiologist Research Physicist Guest Worker Staff Fellow LB NINCDS LB NINCDS LB NINCDS LB NINCDS
COOPERATING UNITS (if any): J. Henderson, Med. Off., Natl Naval Med. Ctr.; R. Latorre, Asst. Prof., Univ. of Chicago; R.J. Lipicky, Prof., Univ. of Cincinnati, Ohio		
LAB/BRANCH Laboratory of Biophysics		
SECTION Section on Molecular Biophysics		
INSTITUTE AND LOCATION IRP, NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.4	OTHER: 0.6
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>It is the purpose of this project to better understand the mechanism of the ionic conductances in membranes which are voltage dependent, i.e., excitable. One of the methods is to study the effect of chemicals which interact with ionic channels, in order to find out more about the channels. In particular, we want to learn where the voltage-dependent gates are located and how the gates respond to electric fields. Currently, we are studying <u>yohimbine</u> which changes the <u>voltage-dependent sodium conductance</u> in the <u>membrane</u> of the <u>squid giant axon</u>. We are especially interested in the site of yohimbine <u>binding</u> to the membrane.</p> <p>Other studies designed to learn more about the mechanism of voltage-dependent conductance are:</p> <ol style="list-style-type: none"> 1) The effect of <u>pressure</u> on axonal and <u>synaptic membranes</u> of the <u>squid</u>. 2) The effects of membrane potential on the <u>calcium</u> channel in the <u>barnacle muscle membrane</u>. 		

Project Description

The object of this project is to try to understand the mechanism of the ionic conductances which are voltage dependent.

We have studied the effects of yohimbine, an indole alkaloid. Yohimbine causes decreases in action potential amplitudes when repeatedly pulsed. Sodium currents were measured in the squid giant axon using the voltage-clamp technique. When depolarizing test pulses were at least two minutes apart, there was a decrease in the sodium current associated with no voltage or time constant changes. This effect we have termed the tonic effect. Although yohimbine acts both internally and externally, we have determined that its site of action is on the inside. When depolarizing test pulses, of short duration, at frequencies of one hertz or greater were used, there was a further reduction in sodium currents. The magnitude of this further reduction was increased for increasingly depolarized test pulses and was decreased for increasingly hyperpolarized prepulses. The time to reach a final steady sodium current was decreased by increasing either the depolarization of the test pulses or the hyperpolarization of the prepulse. This voltage-dependent effect is not consistent with an electric field effect on the binding constant of the drug with its site. We plan to study more carefully this voltage-dependent effect by decreasing the test pulse durations instead of altering the frequencies of stimulation.

Experiments are also being conducted on the effect of yohimbine on the passive sodium uptake on cultured neuroblastoma cells to determine the site of membrane binding. Other workers have shown that batrachotoxin (BTX) causes an increase in the passive sodium uptake and that it is probably due to an activation of the sodium channel. Yohimbine inhibits this BTX effect. Experiments are planned to determine whether this inhibition is competitive or non-competitive.

We have previously shown that increased helium pressure slows down the action potential and the voltage-clamped sodium and potassium currents in the squid giant axon. Further experiments altering the prepulse conditions did not exhibit a pressure effect on the sodium inactivation process. We also studied the squid giant synapse under high helium pressure. The pre-synaptic nerve was stimulated extracellularly and the post-synaptic potential was recorded using an insulated platinum wire exposed at its tip and placed longitudinally down the axon to the synapse. With repetitive stimulation, the synapse fatigues more easily. Thus, at 200 atmospheres the number of action potentials before complete fatigue occurs is only about 5% of the number when there is no helium pressure. Since a high pressure neurological syndrome occurs in man exposed to pressures greater than twenty atmospheres, experiments using pressure on excitable membranes might be able to explain some of the effects of this syndrome.

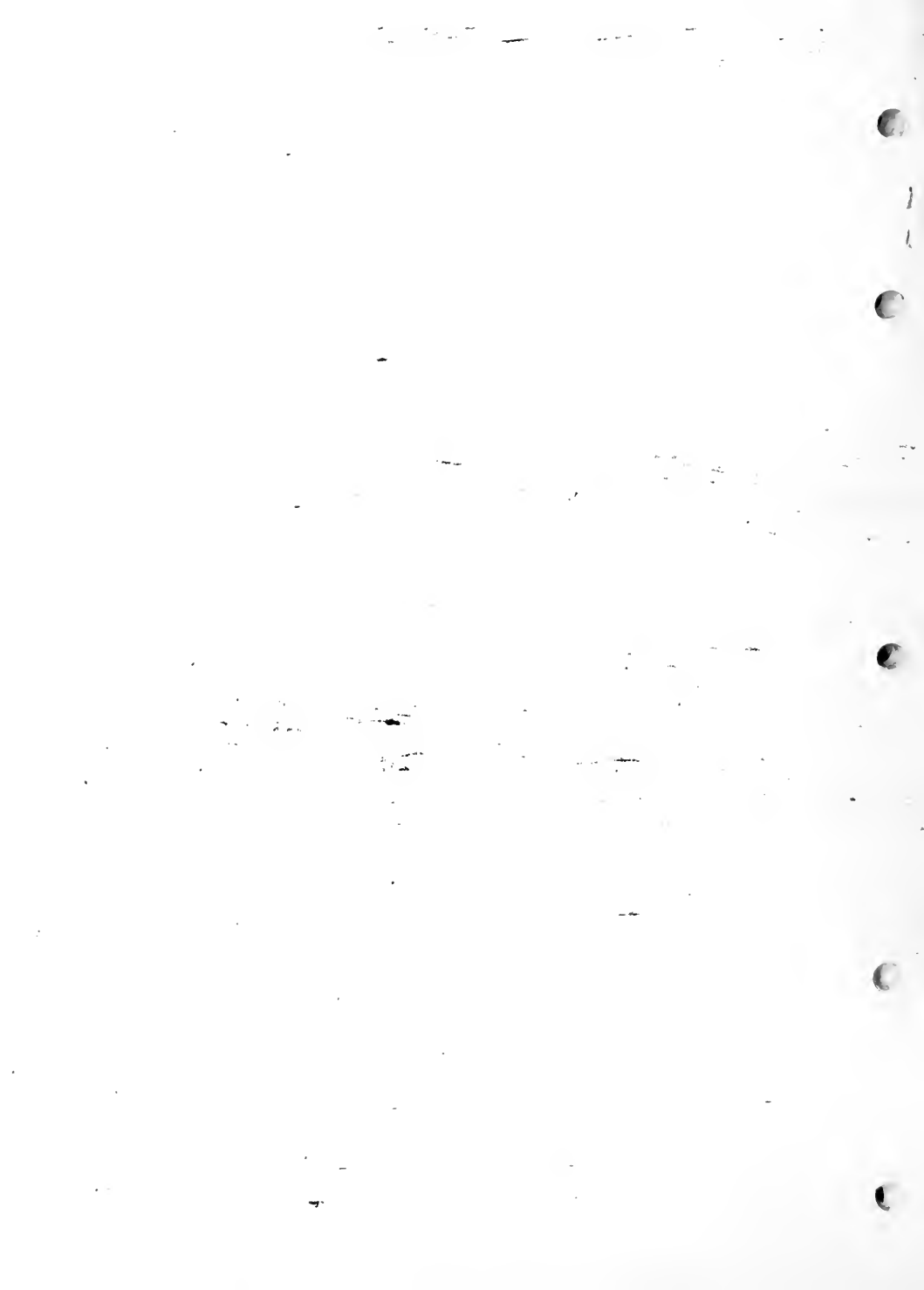
Z01 NS 02218-01 LB

We also plan to test how inhibitors of calcium current (such as verapamil) influence the voltage-clamped calcium currents in the membrane of the barnacle muscle. In particular, the site of action of the inhibitors will be studied. We are especially interested in whether these agents block calcium channels or affect the gates of the channels.

Publications:

Ehrenstein, G., Gilbert, D.L., and Lipicky, R.J.: Does phospholipid flip-flop affect axon potassium channels? Biophys. J. 15: 847-849, 1975.

Henderson, J.V., Jr. and Gilbert, D.L.: Slowing of ionic currents in the voltage-clamped squid axon by helium pressure. Nature 258: 361-362, 1975.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02219-01 LB																
PERIOD COVERED July 1, 1975 to June 30, 1976																		
TITLE OF PROJECT (80 characters or less) Structure and function of the perineurium																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0"> <tr> <td>PI:</td> <td>R. E. Taylor</td> <td>Superv. Res. Physiologist</td> <td>LB NINCDS</td> </tr> <tr> <td>Other:</td> <td>L. Firouzi</td> <td>Visiting Fellow</td> <td>LB NINCDS</td> </tr> <tr> <td></td> <td>S. I. Rapoport</td> <td>Medical Officer, Researcher</td> <td>LNP NIMH</td> </tr> <tr> <td></td> <td>M. W. Brightman</td> <td>Research Physiologist</td> <td>LNNS NINCDS</td> </tr> </table>			PI:	R. E. Taylor	Superv. Res. Physiologist	LB NINCDS	Other:	L. Firouzi	Visiting Fellow	LB NINCDS		S. I. Rapoport	Medical Officer, Researcher	LNP NIMH		M. W. Brightman	Research Physiologist	LNNS NINCDS
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	S. I. Rapoport	Medical Officer, Researcher	LNP NIMH															
	M. W. Brightman	Research Physiologist	LNNS NINCDS															
COOPERATING UNITS (if any) Laboratory of Neurophysiology, NIMH; Laboratory of Neuropathology and Neuroanatomical Sciences, NINCDS																		
LAB/BRANCH Laboratory of Biophysics																		
SECTION Section on Molecular Biophysics																		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014																		
TOTAL MANYEARS: 1.1	PROFESSIONAL: 0.9	OTHER: 0.2																
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to determine how and to what extent the <u>perineurium</u> is involved in the maintenance and regulation of the ionic and <u>metabolic</u> environment of the axons of peripheral nerves. The topics of interest include: (1) <u>passive permeability</u> to electrolytes and non-electrolytes; (2) <u>active transport</u> of ions; (3) <u>facilitated diffusion</u> or transport of amino acids and glucose; (4) <u>electrical impedance</u> and short circuit currents; (5) the determination of the normally existing composition of the <u>extracellular fluid</u> in the <u>endoneurium</u> .																		

Project Description:

The extracellular space in the endoneurium of peripheral nerves is isolated by the endothelial lining of cell capillaries and by the single layer of cells in the perineurium which are connected together by tight junctions. This project is concerned with the study of the role of the epithelial cell layer in the perineurium.

The methods employed are principally the standard techniques used to study unidirectional fluxes of various substances across the isolated and perfused perineurial sheath of the frog or toad, including the use of radioactive tracers. In addition, histological techniques are employed in collaboration with specialists in electron microscopy, and electrical measurements are made using internal and external voltage and current supplying electrodes.

This is a new project and the only major findings of note are the confirmation of early unpublished results of the principal investigator on the impedance of the isolated sheath.

The blood-nerve barrier bears the same relation to peripheral nerve axons as the blood-brain barrier does to the brain. Very little work has been done in this area. It is not known, for example, how the composition of the fluid in the extracellular space compares with cerebrospinal fluid. It would appear that the barriers in the perineurium would be intimately involved in a number of functions of the axons and in a variety of peripheral neuropathies.

The proposed course of this project is to begin to explore the passive permeability, active transport, facilitated diffusion and electrical impedance of the perineurium and to relate these to structural features as revealed by electron microscopy.

Annual Report

July 1, 1975 through June 30, 1976

Laboratory of Experimental Neurology, Intramural Research Program
National Institute of Neurological and Communicative Disorders and Stroke

William F. Caveness, M.D.

General Statement

This is a small laboratory with five/four permanent positions. Its productivity is dependent upon the conceptual and technical competence of each of its staff, the effective contract support for processing anatomical specimen and supply of newborn monkeys, and the collaboration of scientists at Harvard University, and at other branches and laboratories at NIH.

This activity is a continuation of a primate laboratory established at Columbia University in 1958 as an adjunct to the Seizure Clinic for Children, Columbia Presbyterian Medical Center, both under my direction. Its initial purpose was to better understand clinical seizure patterns in infants and young children by studying the evolving seizure patterns in the developing brain of the monkey. The first eight years of this investigation is summarized in *Ontogeny of Focal Seizures*, chapter 19, in *Basic Mechanisms of the Epilepsies*, Little, Brown and Co., 1969.

In 1961, in collaboration with Brookhaven National Laboratories, a series of studies were begun on the delayed effects of x-irradiation on the cerebral cortex of the *Macaca mulatta*. This was prompted by the clinical observations on the wards of the Neurological Institute of New York of delayed spinal cord necrosis that followed in a matter of months the unintentional exposure of the cord during irradiation therapy for neoplasms of the nasopharynx, lung or mediastinal structures. The results of the first six years of these investigations are set forth in *Pathogenesis of X-irradiation Effects in the Monkey Cerebral Cortex*, *Brain Research*, 1968, 7: Special Issue.

Following the Korean Conflict, there began studies of sequelae in men who were seen by me at the time of their head injuries, in combat or support activities. These findings are summarized in Chapter 15, Vol. 15, *The Epilepsies* and Chapter 25, Vol. 24, *Injuries of the Brain and Skull Part II*, *Handbook of Clinical Neurology*, North Holland Publishing Co., Amsterdam, 1974 and 1976, respectively.

The Laboratory was established within the Intramural Program, NINCDS, NIH, April 1969 with the physical transfer of records and equipment from New York to Bethesda in March 1970.

Current Scientific Effort:

1) Experimental Focal Seizures in the Monkey.

(a) Ontogeny: Earlier electrographic and clinical findings, indicative of the dependence of the seizure pattern on regional organization in the central nervous system, led us to postulate that while the cerebral cortex in the newborn is able to support focal paroxysmal activity, it is dependent upon the subcortical somatotopically linked nuclear masses and circuits for the full development of focal abnormality, its propagation to other parts of the cortex and to a common final pathway that results in the clinical seizure. In the 24 month old (pubescent) monkey, on the other hand, the propagation is under the control of cortical circuitry. To test this concept, two experimental procedures were carried out just prior to penicillin induced focal activity in the motor face area of the right cerebral cortex. By undercutting the site of activation, the propagation was blocked in the newborn and unimpeded at 24 months of age. Interrupting the intracortical connections by circumsecting the activation site had negligible effect in the newborn, but blocked the propagation at 24 months. These findings support the concept that the effective neuronal organization for the propagation of focal paroxysmal activity shifts from subcortical to cortical with maturation. The anatomical substrate in the cerebral cortex for the described seizure activity has been extensively investigated with particular attention to the developing fiber systems, as delineated by Nauta techniques, and the developing pyramidal and stellate cells as demonstrated in Golgi preparations. At birth, cortico-cortical connections are poorly developed, and cortico-subcortical circuits are well demarkated. In contrast, cortico-cortical connections are prominent at 24 months of age. This increase in cortical connectivity is reflected in the postnatal architectonic development of layers II and III, the loci of the most dramatic events of the postnatal development. These studies have been completed and the results published.

(b) Subcortical Factors:

Blood Flow Studies: A logical extension of the preceding observations is a search for the subcortical circuits and nuclear masses that are involved in the propagation of the seizure activity. Previous studies by others have attempted to define "preferential" pathways by depth recording or by selective destruction of subcortical structures. For us it seemed wise to gain a global appraisal prior to the definitive study of individual elements. For this we have employed the [^{14}C] antipyrine technique for the determination of regional cerebral blood flow during selected phases in the propagation. The assumption, based on sound observations by others, is that an increase in neuronal activity will be reflected by an increase in blood flow.

With the cooperation of the Laboratory of Cerebral Metabolism, NIMH, we have developed the [^{14}C] antipyrine method and added modifications that permit the sectioning of the whole head for the autoradiograms and the magnification by five of the radiograms for optical density measurements. This prevents distortion in the brain sections and facilitates densitometric measurement of the isotope uptake in small subcortical nuclear

masses, respectively. In brief: The diffusable radioactive tracer, 200 $\mu\text{C}/\text{kg}$ body weight is infused over a sixty second period during the development of a focal seizure. The monkey is then decapitated and the head immediately immersed in a Freon bath chilled to -100°C by liquid nitrogen. In short the fit is frozen in the brain. Subsequently the brain, in its case, is serially sectioned at 30 micra with a PMV Cryo-microtome at a temperature of -20°C . Approximately 100 sections from each brain are dried at 60°C and placed on blue sensitive X-ray film for macroautoradiographs. From the determination of the concentration of the [^{14}C] antipyrine in the arterial blood and the concentration of the tracer in the macroautoradiographs, the value of the actual regional cerebral blood flow in millilitres per gram per minute is calculated from the formula devised by Kety.

Two advantages in exploring subcortical mechanisms in the newborn are being exploited. First, the level of development and integration of the cortex is such that the better developed subcortical systems are likely to have a clearer expression. Secondly, the propagation of seizure activity, at this age, is protracted in time providing a better opportunity to observe the sequential involvement of neuronal aggregates. In the current series, the seizure activity is being interrupted at two, three and five minutes following the injection of penicillin. In those with the least progression, the electroencephalographic paroxysmal activity has been sporadic and limited to the right sensori-motor area. The accompanying increased blood flow has been limited to the right sensori-motor cortex and the right putamen. With better defined electrographic activity and contralateral clinical phenomena, e.g. face or hand, the ipsilateral increase in blood flow was found in the sensori-motor cortex, supplementary cortex, putamen, globus pallidus, VL, VPL, VPM, substantia nigra and contralateral lobulus simplex of the cerebellum. With further progression of the electrographic expression and contralateral clinical phenomena involving more than one part, e.g. face and hand, the increased blood flow was found in the just described areas, plus the ipsilateral Subthalamic nucleus, Red nucleus, Pontine nucleus, and the contralateral anterior and posterior lobes and nuclei of the cerebellum. This clear cut progression in subcortical structures is set forth in Table 1.

A similar pattern with a faster progression to subcortical structures was found in pubescent and young adult monkeys.

The yield from this appraisal of brain function during the propagation of a focal seizure prompted a refinement that provides a greater resolution in the autoradiograms and a closer approximation to the metabolic state, i.e. the use of ^{14}C -deoxyglucose for the quantitative estimation of the rates of glucose consumption in the various structural components of the brain.

Biogenic Amines: Within the past year we have added an important parameter to the studies of cortical and subcortical structures that are implicated in the propagation of focal seizures. With the Laboratory of Clinical Sciences, Section on Histopharmacology, NIMH, we are examining the changes in neurotransmitters or their enzymes in the areas that have shown increased blood flow. In the routine sectioning of the frozen head, when a structure of interest is exposed, first the section for the autoradiogram is taken,

then a sample of the structure is obtained with an 18-22 gauge needle from the block remaining in the cryostat. The material is immediately transferred to the appropriate solutions for micro-assay of dopamine, norepinephrine, choline acetyl transferase, and glutamic acid decarboxylase. A small numerical ticket marks the defect in the block which is then photographed. These procedures are carried out faster than they can be described with minimal elevation in cryostat temperature. As of April 15, 1976, only three animals have been completed: One control and two with focal seizures. This series will employ young adult monkeys, 3 Kg in weight.

Electrographic Observations: These studies followed the observed prominence of the increased blood flow in the cerebellum during seizure activity. The simultaneous recording of the electrical activity from the right and left cerebral cortex and the right and left anterior lobes of the cerebellar cortex in six 3 Kg animals has demonstrated concomitant paroxysmal activity in the face area of the cerebral motor cortex and the somatotopic representation of the face in the anterior lobe of the contralateral cerebellar cortex. Further, with the advance of the seizure activity to the hand area in the cerebral cortex there is a similar advance to the hand representation in the cerebellar cortex. These electrical phenomena are accompanied by clonic movements in the face and hand, respectively. We plan to exploit this model in two ways: a) the determination of the effect on the cerebellar activity of interrupting the sensory input from muscles and tendons during the focal seizure, by paralyzing the animal prior to the penicillin injection, and b) determining the effect on the propagation of the focal seizure by cooling the paramedian region of the contralateral anterior lobe of the cerebellum.

Significance to Bio-Medical Research and the Program of the Institute: This established model of focal seizures will permit the manipulation of cortical and subcortical activity by pharmacological or physical agents. This should provide new approaches to medical and/or surgical therapy for selected forms of the Epilepsies.

2) Delayed Effects of Ionizing Irradiation on the Brain of the Monkey.

Three modes of brain irradiation in the young adult *Macaca mulatta* have been employed to demonstrate phenomena of clinical interest.

(a) In the first model, the right occipital lobe of 24 animals were exposed to 3,500 rads of orthovoltage radiation, in a single dose. The source was a General Electric Maxitron X-ray machine, operating at 250 KVP and 30 MA. The target to skin distance was 23 cm and the dose rate was 300-350 rad/min. An 0.5 copper filter and an aluminum parabolic filter resulted in an x-ray beam having a half-value layer equal to 2.15 mm of copper.

The early ultrastructural changes were found to be subtle, implicating glycogen metabolism, and perhaps enzyme systems, as reflected by altered lysosomes. With routine histology, and Golgi Cox preparations, the sequential changes prior to 20 weeks were limited to minimal, scattered astrocytic or microglial reactions with occasional

perivascula. collections of mononuclear cells. Around twelve weeks there were detectable though not pronounced alterations in dendritic arbors and to a less extent, a loss in cell bodies. From 20 to 24 weeks, there was a rather abrupt breakdown in neural tissue, represented most clearly in focal areas of myelin destruction, accompanied by proliferative and degenerative changes in astrocytes, microglia and oligodendroglia, and collections of mononuclear cells. Vascular changes included proliferation, occlusion, degeneration and haemorrhagic exudates. Around the periphery were hyperplasia of glia, telangiectasia, and an inflammatory reaction. In the following weeks the focal areas coalesced into wider areas of tissue destruction that were accompanied by reparative processes of varying but incomplete degree.

As a part of this complex lesion, there was a massive break in the blood brain barrier, the clinical implications of which could be profound. To delineate this, an additional set of monkeys received by vein, Evans Blue dye, 2 ml/kg two hours before they were killed. The irradiated right occipital lobe was swollen and deeply stained. The swelling extended from the irradiated region throughout the right hemisphere causing gross brain distortion. To monitor the changes in cerebral spinal fluid pressure, an indwelling catheter reservoir system was employed that permitted sequential measurements of cisternal pressure. In 8 out of 12 irradiated monkeys the pressure curves indicated a delayed, abrupt rise that might take place as early as 18 weeks or as late as 36 weeks following the exposure. The pressure increase achieved an elevation of 3 or more times baseline within a period of two weeks and was reflected in papilledema (4/8), oculomotor palsies (3/8), behavioral blindness (4/8), gross depression in visual evoked response (8/8) and predominately delta activity in the background electroencephalographic activity (8/8). The four animals that developed behavioral blindness are of special interest. The unilateral destruction of the visual cortex is insufficient to account for this as is the bilateral papilledema, since the blindness and papilledema did not always occur in the same animal. Rather, we believe the blindness to be the result of gross distortion of the visual pathways within the brain, i.e. the swelling in the right hemisphere and the compression in the left, implicating particularly the lateral geniculate bodies. Three of the twelve animals showed only a mild elevation in CSF pressure, never more than twice baseline, between the 20th and 36th week. Observed for 52 weeks, none of these animals showed abnormal clinical signs, although all showed mild depression of the visual evoked response on the right. When killed, all showed some staining with Evans Blue, but less pronounced histological changes at the irradiated site than that seen in the previous group of eight monkeys. A single monkey showed an appreciable but more gradual increase in CSF pressure, three times baseline at 22 weeks with a return to, and below, baseline from the 40th week. There was papilledema from the 18th to 30th week, and absent visual evoked response from the 25th to 30th week, then a gradual recovery of the latter during the remainder of the 52 weeks of observation. When killed, the brain showed no staining with Evans Blue but rather a shrunken fibrotic lesion in the irradiated right occipital lobe. The lateral geniculate body on the right showed cell loss involving all lamina, evidently the remnant of pronounced edema in the right hemisphere.

As an extension from the observations of this model we determined the regional cerebral blood flow (rCBF), by the [^{14}C] antipyrine method for evidence of metabolic

impairment, not only within the developing lesions, but throughout the brain. For this, one monkey was killed at baseline, three during the phase of development of increased CSF pressure, one at its peak, and one upon recovery. Two unirradiated animals served as controls. The findings indicated that as the CSF pressure increased the blood flow decreased. This decrease was at first greater in the right hemisphere, the side of the lesion, and in the white matter more than the grey matter. However at the highest pressures the blood flow was decreased bilaterally in both white and grey matter. Of perhaps grave significance was the only partial recovery in blood flow in the monkey whose intracranial pressure had returned to normal. While there was greater improvement in grey than white matter, there remained a diffuse reduction in blood flow suggesting a long term impairment in cellular metabolism and/or blood flow regulatory mechanisms.

The principles demonstrated by this first mode of irradiation, i.e. a portion of the brain with a single dose of 3,500 rads, are: 1) There is a protracted delay, in weeks or months, before there is overt breakdown of neuronal tissue. 2) Once this starts the destructive process proceeds quickly. 3) A prominent feature is a break in the blood brain barrier, that not only contributes to the local destruction, but through the migrating plasmatic fluid can damage remote areas, either directly through separation and compression of neuronal elements, or indirectly through distortion of brain structures and impairment of CSF circulation. 4) The lesions seldom, if ever, heal, in the time span studied. In those instances in which the reparative processes "seal" off the lesions, there may remain focal, and perhaps diffuse, impairment in neuronal function as a remnant of the destructive processes.

(b) In the second model the whole brain was subjected to a single exposure of supervoltage irradiation. Three groups of four monkeys each received 1,000, 1,500 and 2,000 rads, respectively. The source was a linear accelerator with an electron beam energy of 20 MeV, that after collimation was converted to photons by tantalum foils and flattened by a lead disc to provide a nearly constant radiation field. The dose rate was 200 rads/min. The source axis distance was 180 cm.

Those with the whole brain exposed to 1,000 rads in a single dose demonstrated no clinical signs. Those irradiated with 1,500 rads showed progressive neurological abnormalities, including diminished motor activity, myoclonic jerks, high amplitude slow waves (delta activity) in the background EEG, papilledema and loss of visual acuity. With whole brain exposure to 2,000 rads, none of the monkeys survived beyond 26 weeks. Their deteriorating condition included crippling loss in motor power and dexterity with finally an inability to ingest food. Three out of the four, had pronounced papilledema beginning in the 8th week, the fourth, had blurred disc margins in the 12th week, that was less evident at the time of sacrifice.

The brains of those exposed to 1,000 rads showed no abnormality. The brains of those subjected to 1,500 rads, that were killed at or beyond 52 weeks showed evidence of swelling and symmetrically dilated ventricular systems. When sectioned, the brain of the monkey killed at 26 weeks, showed scattered focal lesions in the forebrain white

matter. These consisted of discrete, minute areas of necrosis, in some of which there were mineral deposits. The monkey killed at 52 weeks, showed wide spread areas of coalescing necrosis involving primarily the white matter of the cerebral hemispheres, along with focal lesions in the brain stem and cerebellum. In the focal lesions there was some mineralization with very little found in the confluent areas of necrosis. Vascular changes included amorphous, subendothelial deposits in the small arteries, and capillary telangiectasia. The monkey killed at 78 weeks, had a pattern of confluent and focal necrosis similar in kind, but less in degree than that shown in the monkey killed at 52 weeks. The monkey killed at 102 weeks, on gross examination showed atrophic as well as oedematous changes, along with cerebellar tonsillar herniation. The histological examination of this brain is not yet complete. With whole brain exposure to 2,000 rads, the most striking gross abnormality in all four brains was the symmetrical enlargement of the ventricular system. Upon sectioning, they all had a wide scatter of focal necrotic lesions that, though very high in number had not yet shown coalescing or confluence. The large number of fresh lesions in the brain stem undoubtedly contributed to the inability to survive. Table 2.

The principles to be derived from this second model are straightforward: 1) Whole brain exposure to 1,000 rads in a single dose causes no lesions in the first 104 weeks following irradiation. 2) Exposure to 1,500 rads causes small focal areas of necrosis in the forebrain white matter at 26 weeks, but a much more extensive involvement at and beyond 52 weeks that includes confluent areas of necrosis in grey and white matter, with focal lesions extending into the brain stem. At and beyond 52 weeks there is ventricular dilation, evidently from brain loss. 3) Exposure to 2,000 rads causes such a wide scatter of focal areas of necrosis throughout the brain, including a large number in the brain stem, that survival beyond 20-26 weeks is not possible. All showed atrophic gyri and enlarged ventricular systems.

(c) In the third model, the whole brain was subjected to fractionated exposures of supervoltage irradiation, at approximately 200 rads a day five days a week, with the same equipment described for the previous series. To bracket the human exposure of 6,000 rads, now used for malignant gliomas in several medical centers in the United States, three groups of four monkeys each received 4,000, 6,000 and 8,000 rads in courses of 4, 6, and 8 weeks, respectively.

The monkeys exposed to 4,000 rads showed no functional abnormality. Those that received 6,000 rads showed alteration in visual evoked response and/or papilledema from the 8th week following irradiation, the latter usually receding between the 16th and 24th week. From the 32nd week high amplitude slow waves were apparent in the EEG. Those that received 8,000 rads exhibited a course not unlike those that received 6,000 rads up to 26 weeks. Beyond that time there was a progressive deterioration reflected in severe impairment in motor function, papilledema followed by optic atrophy and behavioral blindness, and prominent delta activity in the EEG.

The animals that received 4,000 rads had no structural abnormalities. In those exposed to 6,000 rads, the one killed at 26 weeks, showed 143 lesions scattered throughout the forebrain, predominantly in the white matter of the corona radiata, centrum

semiovale and internal capsule, with a fair number in the central grey matter. These consisted of areas of necrosis, 1 mm or less in diameter, attended with varying degrees of vascular, glial and macrophage reactions. The individual lesions were of different ages. The most recent consisted of a central core of necrotic brain surrounded by a narrow rim of macrophage response. Slightly older lesions contained numerous macrophages throughout. The oldest showed mineral deposits that stained for both calcium and iron. Myelin sections adjacent to the Nissl sections at times revealed corresponding areas of palor that often exceeded in extent the area of necrosis, suggesting focal "vasogenic" edema. Adjacent to the punctate lesions were occasional grossly altered vascular endothelial cells. Quite apart from these, were areas of capillary proliferation, or telangiectasia, 5-8 mm in extent. At 52 weeks, there were only 12 small necrotic lesions, and most of these were mineralized. In addition there were innumerable minute areas of mineral deposits that may have represented remnants of larger necrotic lesions, or degenerated vascular walls or plasma products. A more active process was reflected in widespread areas of dilated capillaries or telangiectasia, more than were seen at 26 weeks. At 66 weeks there were 63 small necrotic lesions with varying degrees of associated reactions including mineralization in 49. There were also scattered minute mineral deposits bilaterally. At 78 weeks there was a wide distribution of 242 focal necrotic lesions with a somewhat greater extension into the brain stem than was seen in the animals that were killed at shorter intervals. The accompanying vascular reactions and mineral deposits were, however, more apparent. From the preceding it appears that in the monkeys exposed to 6,000 rads there is a tendency to fewer and fewer necrotic lesions to appear with the passage of time with a progressively larger number that are mineralized, suggesting a tendency toward healing. The monkey killed at 78 weeks showed more lesions than were found at earlier intervals, but few of these were recent, sustaining the impression of a trend toward repair.

The effects from 8,000 rads at 26 weeks, e.g. 64 focal lesions, were similar in kind but greater in degree, than those from 6,000 rads. One monkey killed off schedule at 32 weeks, showed 2,089 focal lesions throughout the white and grey matter of the brain, with 115 in the brain stem. At 52 weeks there was a confluence of necrotic lesions in the swollen cerebral hemispheres including thalamus and basal ganglia, but fewer focal lesions in the brain stem than were found in the monkey that was incapacitated at 32 weeks. At 78 weeks there was massive brain destruction from confluent necrotic lesions in the cerebral hemispheres. The loss in brain substance was reflected in the atrophy of the gyri and marked enlargement of the ventricular system. These monkeys exposed to 8,000 rads demonstrate with the passage of time a consistent, progressive increase in number and confluence of necrotic lesions. Even within this relentless course the monkey killed at 78 weeks showed a somewhat less active and more atrophic process than the monkey killed at 52 weeks. Table 3.

From this third model, employing fractionated exposure to high energy irradiation, the following implications may be considered: 1) The extent of the delayed effects is dose dependent. The effects from 4,000 rads are negligible; from 6,000 rads, considerable; from 8,000 rads, profound. 2) The resultant necrotic lesions from 6,000 rads are small, widely scattered, with a predilection for the forebrain white matter but not excluding the central grey matter and brain stem. The possibility of focal as well as general clinical

phenomena is inherent in this kind of brain damage. 3) The time course is important. Not only is there a delay of weeks or months before there is overt tissue breakdown but the individual lesions, evidently begin their cycle at different times, with the probability of their overall influence extending over a protracted period. Although there is an apparent trend toward recession in numbers of fresh necrotic lesions, adequate repair may be delayed or never occur. The effects at one year from 6,000 rads include considerable mineralization of the necrotic lesions, but a far greater extent of telangiectasia than found at six months. 4) At one phase in the developing areas of necrosis there may be multiple minute breaks in the blood brain barrier that when sufficient in number cause diffuse brain swelling, reflected by papilledema. 5) The lesions from 8,000 rads at six months are not strikingly different from those following 6,000 rads, however, by twelve months the extensive and coalescing lesions from the former produce gross brain destruction.

Current and future activities: A definitive series was started in July 1975 that will more closely simulate the human therapeutic experience and provide more precise information regarding the pathogenesis of the radiation lesions. Twenty-one adult male monkeys, 8 to 12 kilos in weight, were selected to more closely approximate in their age the adult humans that are irradiated for malignant gliomas. The brains of these animals were exposed to 6,000 rads of supervoltage radiation in a six week course, with alternate sides of the head exposed on alternate days. They are being killed, in sets of three, at sequential intervals following the beginning of the radiation course, to gain a better concept of the development and degree of recession of the lesions produced by this mode of irradiation. Their clinical course is being monitored by neurological observations, funduscopy photography, and spinal fluid examination for protein and lactic dehydrogenase. To these regularly scheduled examinations there will be added computerized axial tomography, that is expected to be available in May 1976. The latter should not only provide evidence of brain loss as reflected in dilated ventricles but, with the use of enhancement techniques, evidence of breaks in the blood brain barrier that would give a dynamic appraisal of the location and degree of tissue breakdown, and repair.

Significance to Bio-Medical Research and the Program of the Institute: The principal advantage in simulating a therapeutic radiation regime in a monkey model is to observe the effects in a brain uncomplicated by pre-existing pathology, e.g. that resulting from a neoplasm, surgical trauma or chemotherapy. This kind of information will be useful in the planning of therapeutic efforts in man, with attention to "risk-benefit" factors, and in the interpretation of fresh neurological findings following therapy. There is a direct relevance to a major program of the Cancer Institute, i.e. the Brain Tumor Study Group, and we are privileged to share in their findings as this group is made aware of our results.

3) Structural and Functional Sequelae of Penetrating Head Injury, Phase I.

A registry for Head and Spinal Cord Injuries, as they occurred in military combat in Vietnam, was developed at the request of the Surgeon General of the U.S. Navy and was implemented with the cooperation of the Surgeons General of the U.S. Army and

U.S. Air Force. The purpose was to insure uniformity of data collection and to identify cases for present and future studies. The yield from field surgeons, 1967 to 1970, was 2,043 entries that included 1,683 head injuries, 329 spinal cord injuries and 31 combinations of the two. The average age at time of injury was 21.6 years. A rigorous appraisal demonstrated the uniformity and completeness of 1,540 head injury forms. Ninety per cent of these were the result of explosively propelled missiles, 10% the result of vehicular accidents, falls, or blunt objects. In 85% there was local brain destruction, as evidenced by penetration of the dura with cortical laceration. Sixty per cent of those were accompanied by immediate loss in consciousness, by history. Thirty per cent were in coma responding only to pain at time of examination, that was generally within six hours of injury. This provides an opportunity to group selected cases by regions of focal damage, with and without alteration in consciousness. These categories constitute a background against which the natural history of sequelae may be determined.

A contract project entitled, "Structural and Functional Sequelae of Penetrating Head Injury, Phase I", was submitted to NINCDS in May 1975. A Feasibility Study for Phase I was approved in March 1976, and will be implemented in May 1976. The conduct of this plan will update the clinical data on 1,500 selected cases from the Registry, by a review of the accumulated Military and Veteran Administration hospital records since the time of injury. The assembly of the records, abstracting the pertinent data, coding, transfer to magnetic tape, and analyses of sequelae, so identified, will be carried out in cooperation with the Medical Follow-up Agency of the National Research Council, NAS, the Physical Sciences Laboratory of the Division of Computer Research and Technology, NIH, and three specially qualified professionals at the Eastern Virginia Medical School, Vanderbilt University Medical School and Harvard Medical School, respectively. Concomitant with the completion of Phase I will be the planning of Phase II, i.e., an in-hospital examination of three quarters of these head injured casualties with techniques not previously available, including computerized axial tomography for the determination of brain loss. A second project proposal will be required for Phase II.

Significance to Bio-Medical Research and the Program of the Institute: The observations during the acute phase of injury in these cases are more uniform and probably more accurate than any previous series of comparable size. This provides an extraordinary opportunity for studies of prognostic factors and the natural history of disabilities in central nervous system trauma in man. The recent development of new techniques for evaluating functional deficits, e.g. regarding language and memory and new techniques for determining alterations in brain structure e.g. the CAT Scan, will afford an unique opportunity in Phase II for fresh insight into posttraumatic sequelae and into the function of the remaining brain.

Table 1. rCBF Pattern with Progression of Focal Seizures

Monkey #	Interval	EEG	Clinical	Cerebral Cortex			Basal Ganglia					Thalamus						Cerebellum					Brain Stem				
				Motor	Sensory	Sup. Motor	Caudate	Putamen	G.P. (L)	G.P. (M)	Subth. N.	Subst. Nigra	Red N.	VA	VL	VPL	VPM	CM	MD	Ant. Lobe	(Lob. Simpl.)	Post. Lobe	(Paramed. L.)	Dentate N.	Interpos. N.	Fastig. N.	Pont. N.
N-19	2'																										
N-25	2'																										
N-27	2'		Spike (R)	x	x																						
N-28	3'			x	x																						
N-5	5'			xx	xxx																						
N-26	2'		Spike (R)	xx	xxx																						
N-31	3'		Face (L)	x	x	xx	xx	xx	xx	x																	
N-35	3'			x	xx																						
N-9	5'		Spike (R)	xx	x	xxx	xxx	xxx	xxx	xxx	xxx	x			xxx	xx	x										
N-23	5'		Face (L)	xxx	xxx	xxx	xx	xxx	xx	xxx	x	xx			xx	xx											
N-12	5'			xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	x		xxx	xxx											
N-29	3'		Spike (R&L)	xx	xx	xx	xx	xx	xx	xxx	xxx	xx	x		x	x											
N-11	5'		Face &	xx	xxx	xxx	xx	xxx	xxx	xxx	xxx	xxx			x	xxx	x										
N-24	5'		Hand (L)	xxx	xxx	xxx	xx	xx	xxx	xxx	xxx	xx			x	xxx	xxx	xxx	xxx	xxx	xxx	xx	x	x	x	x	
N-22	5'			xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx		x	xx	xxx	xxx	xxx	xxx	xxx	xx	xxx	x	x	x	
N-4	-		Control																								
N-6	-		Control																								

Unilateral increase of rCBF: x = 20-30%, xx = 30-50%, xxx = 50% +

Table 2. Distribution of focal lesions after single exposure

Structure	1500 rads				2000 rads			
	26 wks	52 wks	78 wks	102 wks	19 wks	20 wks	20 wks	26 wks
	L-32	L-31	L-30	L-33	L-36	L-35	L-37	L-34
Cerebral Cortex		Confluent	5					
Corona Radiata	31	Confluent	Confluent		303	627	122	669
Centrum Semiovale	32	Confluent	Confluent		213	406	125	446
Corpus Callosum		Confluent	2		6	67	12	55
Fornix		4				20	10	7
Internal Capsule		Confluent	18		65	53	21	66
Thalamus		Confluent	8			4	22	22
Hypothalamus		2			12	3	12	37
Subthalamus		Confluent			6	2	1	7
Optic Tract					4	2	8	16
Basal Ganglia		Confluent	3		12	1	3	15
Amygdala					1		6	
Archicortex		Confluent			4	14	16	46
Tectum		12			2			4
Tegmentum, Mesencephalon		57	10		53	27	49	68
Tegmentum, Pons		23	4		46	39	38	120
Basis Pontis		13			95	95	108	150
Medulla		2	1		49	22	12	25
Cerebellar White Matter		52	12		27	79	49	73
Cerebellar Roof Nuclei		3	1		29		1	1

Total	63	(168)	(74)		927	1,461	615	1,827
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Table 3. Distribution of focal lesions after fractionated exposure

Structure	6000 rads					8000 rads				
	26 wks	52 wks	L-19	L-14	L-16	26 wks	32 wks	52 wks	78 wks	
	L-13	L-13	L-19	L-14	L-16	L-20	L-22	L-23	L-21	
Cerebral Cortex	2				3		3	Confluent	Confluent	
Corona Radiata	93			46	176	16	956	Confluent	Confluent	
Centrum Semiovale	13	5		13	22	19	593	Confluent	Confluent	
Corpus Callosum	1					1	24	Confluent	Confluent	
Anterior Commissure	1									
Fornix						1	35	7		
Internal Capsule	14	3		3	2	5	34	Confluent	Confluent	
Thalamus	11			1	26		68	Confluent	Confluent	
Hypothalamus						2	14	3		
Subthalamus						1	21	6		
Optic Tract	2	1					1	1		
Basal Ganglia	4	1					20	Confluent	1	
Amygdala							1			
Archicortex							19			
Tectum	1				1		18	11	10	
Tegmentum, Mesencephalon					3	1	84	33	36	
Tegmentum, Pons		2			2	6	57	21	12	
Basis Pontis	1				2	11	35	24	13	
Medulla							21	2	2	
Cerebellar Cortex									16	
Cerebellar White Matter					5	1	80	31		
Cerebellar Roof Nuclei							5	10		
Total number of focal lesions	143	12		63	242	64	2,089	(149)	(90)	

Administrative Constraints: At the outset, this Laboratory was allocated five permanent positions. Two of these have been used for Visiting Associates or Visiting Scientists, that are invited for two year periods. Within the last year, first a "freeze" on new appointments followed by a reduction in "ceiling," i.e., from five to four permanent positions, caused a gap of four and one-half months when the Laboratory was inadequately staffed for the supervision of the new irradiation series and for the neuro-surgical procedures required for the studies in focal seizures. While no deficits occurred, this placed an excessive burden on the remaining members of the Laboratory, and delayed the analyses and publication of completed work. Temporary relief has been afforded by the appointment of a previously selected, specifically qualified Visiting Associate for one year. However, the inability to reappoint this man in August, 1976, for a second year, will again impose a critical handicap to the productivity of this Laboratory.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 2158-02-LEN
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PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Subcortical Factors in Experimental Focal Seizures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W.F. Caveness	Chief, Lab. of Exp. Neurology	LEN NINCDS
OTHER:	M. Kato	Visiting Scientist	LEN NINCDS
	S. Hosokawa	Visiting Fellow	LEN NINCDS
	• S.G. Speciale, Jr.	Research Pharmacologist	LEN NINCDS
	Y. Gottesfeld	Visiting Scientist	LCS NIMH
	D. M. Jacobowitz	Chief, Sect. on Histopharmacology	LCS NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Sciences, Section on Histopharmacology, NIMH

LAB/BRANCH

Laboratory of Experimental Neurology

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

3.0

PROFESSIONAL:

1.8

OTHER:

1.2

SUMMARY OF WORK (200 words or less - underline keywords)

Focal seizures are created in monkeys, at birth and at pubescence, by injecting 25,000 units of penicillin into the right face-hand area of the cerebral motor cortex. The development and propagation of the seizure is monitored by electroencephalography, electromyography and clinical observations. At selected intervals during the propagation of the focal seizures the animal is decapitated, the head frozen and the active subcortical neuronal aggregates are delineated by an increase in the regional cerebral blood flow as determined by the ^{14}C antipyrine method. The structures so identified are sampled for microassay of neurotransmitters or their enzymes.

Project Description

Objectives: To better understand the subcortical neuronal aggregates that are critical to the propagation of focal paroxysmal activity originating in the sensori-motor cortex.

Background: In the established animal model, focal seizures are induced by injecting 25,000 units of penicillin in 0.025 cc of aqueous solution 3 mm into the face-hand area of the right motor cortex of the Macaca mulatta. The onset and elaboration of the attack pattern is simultaneously monitored by electroencephalography or electrocorticography, electromyography and clinical observations.

Previous oncogenetic observations, completed in Project No. Z01 NS 01694-07 LEN, stemmed from the postulate that while the cerebral cortex in the newborn is able to support focal paroxysmal activity, it is dependent upon the subcortical somatotopically linked nuclear masses and circuits for the full development of focal abnormality, its propagation to other parts of the cortex and to a common final pathway that results in the clinical seizure. In the 24 month old (pubescent) monkey, on the other hand, the propagation is under the control of cortical circuitry. To test this concept, two experimental procedures were carried out just prior to penicillin induced focal activity in the motor face area of the right cerebral cortex. By undercutting the site of activation, the propagation was blocked in the newborn and unimpeded at 24 months of age. Interrupting the intracortical connections by circumsecting the activation site had negligible effect in the newborn, but blocked the propagation at 24 months. These findings support the concept that the effective neuronal organization for the propagation of focal paroxysmal activity shifts from subcortical to cortical with maturation. The anatomical substrate in the cerebral cortex for the described seizure activity has been extensively investigated with particular attention to the developing fiber systems, as delineated by Nauta techniques, and the developing pyramidal and stellate cells as demonstrated in Golgi preparations. At birth, cortico-cortical connections are poorly developed, and cortico-subcortical circuits are well demarkated. In contrast, cortico-cortical connections are prominent at 24 months of age. This increase in cortical connectivity is reflected in the postnatal architectonic development of layers II and III, the loci of the most dramatic events of the postnatal development.

Current Methods: A logical extension of the preceding observations is a search for the subcortical circuits and nuclear masses that are involved in the propagation of the seizure activity. Previous studies by others have attempted to define "preferential" pathways by depth recording or by selective destruction of subcortical structures. For us it seemed wise to gain a global appraisal prior to the definitive study of individual elements. For this we have employed the [^{14}C] antipyrine technique for the determination of regional cerebral blood flow during selected phases in the propagation. The assumption, based on sound observations by others, is that an increase in neuronal activity will be reflected by an increase in blood flow.

With the cooperation of the Laboratory of Cerebral Metabolism, NIMH, we have developed the [^{14}C] antipyrine method and added modifications that permit the sectioning of the whole head for the autoradiograms and the magnification by five of the radiograms for optical density measurements. This prevents distortion in the brain sections and facilitates densitometric measurement of the isotope uptake in small subcortical nuclear masses, respectively. In brief: The diffusable radioactive tracer, 200 $\mu\text{g}/\text{kg}$ body weight, is infused over a sixty second period during the development of a focal seizure. The monkey is then decapitated and the head immediately immersed in a Freon bath chilled to -100°C by liquid nitrogen. In short the fit is frozen in the brain. Subsequently the brain, in its case, is serially sectioned at 30 μm with a PMV Cryo-microtome at a temperature of -20°C . Approximately 100 sections from each brain are dried at 60°C and placed on blue sensitive X-ray film for macroautoradiographs. From the determination of the concentration of the [^{14}C] antipyrine in the arterial blood and the concentration of the tracer in the macroautoradiographs, the value of the actual regional cerebral blood flow in millilitres per gram per minute is calculated from the formula devised by Kety.

Two advantages in exploring subcortical mechanisms in the newborn are being exploited. First, the level of development and integration of the cortex is such that the better developed subcortical systems are likely to have a clearer expression. Secondly, the propagation of seizure activity, at this age, is protracted in time providing a better opportunity to observe the sequential involvement of neuronal aggregates.

Major Findings: In a series of 15 newborn monkeys the seizure activity was interrupted at two, three or five minutes following the injection of penicillin. In those with the least progression, the electroencephalographic paroxysmal activity was sporadic and limited to the right sensori-motor area. The accompanying increased blood flow was limited to the right sensori-motor cortex and the right putamen. With better defined electrographic activity and contralateral clinical phenomena, e.g. face or hand, the ipsilateral increase in blood flow was found in the sensori-motor cortex, supplementary motor cortex, putamen, globus pallidus, thalamic VL, VPL and VPM, substantia nigra and contralateral lobulus simplex of the cerebellum. With further progression of the electrographic expression and contralateral clinical phenomena involving more than one part, e.g. face and hand, the increased blood flow was found in the described areas, plus the ipsilateral, caudate, thalamic CM and MD, Subthalamic nucleus, Red nucleus, Pontine nucleus, and the contralateral anterior and posterior lobes and nuclei of the cerebellum. This clear cut progression in subcortical structures is set forth in Table 1. A similar pattern with a faster progression to subcortical structures was found in pubescent and young adult monkeys.

The yield from this appraisal of brain function during the propagation of a focal seizure prompted a refinement that provides a greater resolution in the autoradiograms and a closer approximation to the metabolic state, i.e. the use of ^{14}C -deoxyglucose for the quantitative estimation of the rates of glucose consumption in the various structural components of the brain.

Within the past year we have added an important parameter to the studies of cortical and subcortical structures that are implicated in the propagation of focal seizures. With the Laboratory of Clinical Sciences, Section on Histopharmacology, NIMH, we are examining the changes in neurotransmitters or their enzymes in the areas that have shown increased blood flow. In the routine sectioning of the frozen head, when a structure of interest is exposed, first the section for the autoradiogram is taken, then a sample of the structure is obtained with an 18-22 gauge needle from the block remaining in the cryostat. The material is immediately transferred to the appropriate solutions for micro-assay of dopamine, norepinephrine, choline acetyl transferase, and glutamic acid decarboxylase. A small numerical ticket marks the defect in the block which is then photographed. These procedures are carried out faster than they can be described with minimal elevation in cryostat temperature. As of April 15, 1976, only three animals have been completed: One control and two with focal seizures. This series will employ young adult monkeys, 3 Kg in weight.

A third method that is being employed is based on the observed prominence of the increased blood flow in the cerebellum during the seizure activity and consists of the simultaneous recording of the surface electrical activity from the cerebral cortex and the cerebellar cortex during the propagation of the paroxysmal phenomena.

For this fourteen monkeys, 3.0-3.5 Kg, under halothane anesthesia, received bilateral convexity and suboccipital craniectomies. The dura was reflected over the precentral gyri and slit just beneath the transverse sinus. The cerebral cortical electrodes consisted of four pairs of platinum contacts, encased in a thin plastic sheath. Each contact was 1 mm in diameter with 2 mm clearance between the inner edges of each pair. The pairs were separated by 4 mm. The total array was placed adjacent to the right central sulcus, opposite the convexity of the arcuate sulcus, providing 15 mm coverage of the motor strip, i.e. extending from the face-hand area over the representation of arm, trunk, and leg. A similar array of electrodes was slipped beneath the tentorium, providing 15 mm coverage over the left paravermal region of the cerebellum, with its somatotopic representation of face, upper and lower extremities. For ancillary use, comparable electrode arrays were positioned over the left cerebral cortex and the right cerebellar cortex, respectively. Closures were effected by gel foam and scalp clips. Three hours after the completion of the surgical procedures and the end of the anesthesia, the critical observations were conducted with the animal fully alert, in a prone position, the head fixed and the limbs loosely restrained. The focal seizures were induced in the usual manner by the injection of 25,000 units of penicillin into area 4 of the face-hand area of the right motor cortex. Prior to and throughout the development and propagation of the seizures, bipolar recording of the surface electrical activity was obtained, from the indicated pairs of electrodes, with a Grass 8-18 electroencephalograph. Clinical manifestations were monitored by visual observations and by electromyography from the musculature of the left face and extremities. After one hour of data collection, the animal was killed with an excessive dose of pentobarbital and the electrode positions verified.

Results: There was a very close correlation between the initial and subsequent rhythmic spike activity from the right cerebral and the left cerebellar cortices, as evidenced by the following: a) In eleven out of fourteen monkeys, the time of onset of the spike activity in the right cerebral cortex and the left cerebellar cortex was identical, as recorded by the ink-writing equipment. In the remaining three, the lag in the appearance of the first spike from the contralateral cerebellum was 20", 40", and 64" respectively; b) With the cessation of spike activity in the cerebral cortex, there was a simultaneous cessation in the cerebellar spike activity; c) With progression of the focal fit, and the increase in complexity of the cerebral wave form, e.g., bursts of multiple spikes, the same complexity appeared in the cerebellar cortex; d) When the frequency of the paroxysmal activity changed in the cerebral cortex, e.g. a sudden transient shift from 3 Hz to 5 Hz, a precisely similar change took place in the cerebellar cortex; e) With the advent of clinical expression the spike activity in the electromyogram was coincident with the spike activity in both the cerebral and cerebellar cortices. The location of the spike activity was somatotopically related, i.e. the paroxysmal activity in the face-hand area of the precentral gyrus of the right cerebral cortex was coincident with that in the left lobulus simplex, representative of the face in the cerebellar cortex; f) When the paroxysmal activity progressed from the face-hand area of the cerebral cortex to those of arm, trunk, and leg, a comparable progression took place from the contralateral lobulus simplex, representing face, to those parts of the anterior lobe of the cerebellum representing the upper and lower extremities. These phenomena were accompanied by an appropriate contralateral clinical progression from face to upper and lower extremities. In three of the animals in which the penicillin injection was shifted to the leg-foot areas in the motor strip, the somatotopic progression was in the opposite direction, simultaneously in the cerebral and cerebellar cortices, as well as in the clinical expression.

Proposed Course: The energetic pursuit of the combined deoxyglucose and neurotransmitter studies of subcortical structures that are critical to the propagation of a focal seizure. Further, the cerebral-cerebellar electrographic model will be exploited in two ways: a) the determination of the effect on the cerebellar activity of interrupting the sensory input from muscles and tendons during the focal seizure, by paralyzing the animal prior to the penicillin injection, and b) determining the effect on the propagation of the focal seizure by cooling the paramedian region of the contralateral anterior lobe of the cerebellum.

Significance to Bio-Medical Research and the Program of the Institute: The results from these investigations should delineate the principal subcortical factors in the propagation of focal seizures in the monkey and thereby provide fresh approaches to therapeutic efforts in man.

Publications:

Ueno, H., Yamashita, Y. and Caveness, W.F.: Regional cerebral blood flow pattern in focal epileptiform seizures in the monkey. Exp. Neurol. 47: 81-96, 1975.

Caveness, W.F., Ueno, H. and Kemper, T.L.: Subcortical factors in experimental jacksonian seizures. Proceedings of the VIIIth International Congress of Neuropathology, Budapest, September 1-7, 1974, Excerpta Medica, Amsterdam, 1975, pp 29-34.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER <div style="font-size: 1.2em;">Z01 NS 02190-01-LEN</div>
PERIOD COVERED <div style="text-align: center;">July 1, 1975 to June 30, 1976</div>		
TITLE OF PROJECT (80 characters or less) <div style="text-align: center;">Thermal Manipulation of Paroxysmal Neuronal Activity</div>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	W.F. Caveness M. Kato S. Wakisaka	Chief, Lab. of Exp. Neurology Visiting Scientist Visiting Associate <div style="display: flex; justify-content: space-between;"> <div>LEN NINCDS</div> <div>LEN NINCDS</div> <div>LEN NINCDS</div> </div>
COOPERATING UNITS (if any) <div style="text-align: center;">None</div>		
LAB/BRANCH <div style="text-align: center;">Laboratory of Experimental Neurology</div>		
SECTION		
INSTITUTE AND LOCATION <div style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</div>		
TOTAL MANYEARS: <div style="text-align: center;">1.5</div>	PROFESSIONAL: <div style="text-align: center;">1</div>	OTHER: <div style="text-align: center;">0.5</div>
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>In the study of subcortical structures that are brought into play with the propagation of paroxysmal activity from a penicillin focus in the motor cortex of the monkey, a question of importance is: which of these neuronal aggregates are essential to the full development of the <u>experimental focal seizure</u>? In seeking an answer, neuronal blockade of structures with previously demonstrated involvement will be brought about by a stereotactically controlled <u>cryoprobe</u>. The instrument at hand permits graded cooling at its tip, that is 0.1 cm in diameter and monitored by a microthermocouple. By using non destructive degrees of cooling in selected subcortical areas, the objective will be to temporarily interrupt the progression of the paroxysmal activity, thereby demonstrating the importance of these areas in-fit production.</p>		

Project Description

Objectives: To interrupt, in a reversible manner, the paroxysmal activity in cortical or subcortical neuronal aggregates during the propagation of an experimental focal seizure.

Background: The delineation of cortical and subcortical structures that sequentially come into play during the propagation of a focal seizure has been accomplished with the use of ^{14}C antipyrine regional cerebral blood flow studies. It is expected the definition of the role played by these structures will be enhanced by the ^{14}C deoxy-glucose method which should provide quantitative evaluations of glucose utilization. When the latter technique is used in combination with the neurotransmitter studies, as described in Project No.: Z01 NS 02158-02-LEN, a limited number of structures should stand out as of critical importance to the production of the seizure. The structure so identified will be the target for thermal manipulation.

Methods to be Employed: Utilizing the established model of focal seizures induced by the injection of 25,000 units of penicillin in the face-hand area of the right motor cortex, an attempt will be made to prevent or to interrupt the propagation of the paroxysmal activity by cooling selected subcortical structures. Structures that may be considered include the ipsilateral globus pallidus, thalamic VL, VPL or VPM, substantia nigra, and contralateral paravermal aspects of the anterior and posterior lobes of the cerebellum. The mode of locally lowering the temperature is a specifically constructed cryoprobe, 0.1 cm in diameter and 10 cm in length that may be attached to a stereotactic probe carrier for proper insertion into the monkey brain. The cooling is limited to a silver hemisphere, 0.1×0.05 cm, at the tip which is monitored by a microthermocouple. The tip also serves as a monopolar electrode. The gradations in control of cooling at the tip should provide reversible blockade of neural function, expected between 0° and -5° centigrade. This instrument is at hand and operational. It will be put into systematic use in June 1976.

Significance to Biomedical Research and the Program of the Institute: The results from these investigations should effectively supplement the understanding of subcortical factors in the propagation of focal seizures. Further, they should provide a fresh approach to surgical therapy for selected aspects of the Epilepsies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02159-02-LEN
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PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Whole Brain Irradiation within the Therapeutic Range

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. F. Caveness	Chief, Lab. of Exp. Neurology	LEN NINCDS
OTHER:	S. Wakisaka	Visiting Associate	LEN NINCDS
	T.L. Kemper	Lecturer on Neurology, Harvard Medical School	
	D.M. Verrelli	Head, Radiological Physics Dept.	AFRRI

COOPERATING UNITS (if any)

Armed Forces Radiobiology Research Institute
Harvard University

LAB/BRANCH

Laboratory of Experimental Neurology

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

2.3

PROFESSIONAL:

1.1

OTHER:

1.2

SUMMARY OF WORK (200 words or less - underline keywords)

As a part of a continuing series of studies on the delayed effects of ionizing irradiation on the brain of the monkey, the current effort includes sequential observations after whole brain exposure to 6000 rads of supervoltage radiation, in divided doses over a six week period. The protocol simulates as closely as possible that used by twelve medical centers in therapy of malignant gliomas in humans.

Project Description

Background: Previous studies of the delayed effects from 3,500 rads of ortho-voltage radiation, in a single dose, to the right occipital lobe of the young adult *Macaca mulatta* employed three approaches that yielded the information reported in the completed project, Z01 NS 01693-07-LEN.

In brief, the first was concerned with those structural changes that could be detected through the sequential sacrifice of 24 monkeys, in sets of three, over a period of 44 weeks. The early ultrastructural changes were found to be subtle, implicating glycogen metabolism, and perhaps enzyme systems, as reflected by altered lysosomes. With routine histology, and Golgi Cox preparations, the sequential changes prior to 20 weeks were limited to minimal, scattered astrocytic or microglial reactions with occasional perivascular collections of mononuclear cells. Around twelve weeks there were detectable though not pronounced alterations in dendritic arbors and to a less extent, a loss in cell bodies. From 20 to 24 weeks, there was a rather abrupt breakdown in neural tissue, represented most clearly in focal areas of myelin destruction, accompanied by proliferative and degenerative changes in astrocytes, microglia and oligodendroglia, and collections of mononuclear cells. Vascular changes included proliferation, occlusion, degeneration and haemorrhagic exudates. Around the periphery were hyperplasia of glia, telangiectasia, and an inflammatory reaction. In the following weeks the focal areas coalesced into wider areas of tissue destruction that were accompanied by reparative processes of varying but incomplete degree.

As a part of this complex lesion there was a massive break in the blood brain barrier, the clinical implications of which could be profound. To delineate this, an additional set of monkeys received by vein, Evans Blue dye, 2 ml/kg two hours before they were killed. The irradiated right occipital lobe was swollen and deeply stained. The swelling extended from the irradiated region throughout the right hemisphere causing gross brain distortion. To monitor the changes in cerebral spinal fluid pressure, an indwelling catheter reservoir system was employed that permitted sequential measurements of cisternal pressure. In 8 out of 12 irradiated monkeys the pressure curves indicated a delayed, abrupt rise that might take place as early as 18 weeks or as late as 36 weeks following the exposure. The pressure increase achieved an elevation of 3 or more times baseline within a period of two weeks and was reflected in papilledema (4/8), oculomotor palsies (3/8), behavioral blindness (4/8), gross depression in visual evoked response (8/8) and predominately delta activity in the background electroencephalographic activity (8/8). The four animals that developed blindness are of special interest. The unilateral destruction of the visual cortex is insufficient to account for this as is the bilateral papilledema, since the blindness and papilledema did not always occur in the same animal. Rather, we believe the blindness to be the result of gross distortion of the visual pathways within the brain, i.e. the swelling in the right hemisphere and the compression in the left, implicating particularly the lateral geniculate bodies. Three of the twelve animals showed only a mild elevation of CSF pressure, never more than twice

baseline, between the 20th and 36th week. Observed for 52 weeks, none of these animals showed abnormal clinical signs, although all showed mild depression of the visual evoked response on the right. When killed, all showed some staining with Evans Blue, but less pronounced histological changes at the irradiated site than that seen in the previous group of eight monkeys. A single monkey showed an appreciable but more gradual increase in CSF pressure, three times baseline at 22 weeks with a return to, and below, baseline from the 40th week. There was papilledema from the 18th to 30th week, and absent visual evoked response from the 25th to 30th week, then a gradual recovery of the latter during the remainder of the 52 weeks of observation. When killed, the brain showed no staining with Evans Blue but rather a shrunken fibrotic lesion in the irradiated right occipital lobe. The lateral geniculate body on the right showed cell loss involving all lamina, evidently the remnant of pronounced edema in the right hemisphere.

As an extension to this model we determined the regional cerebral blood flow (rCBF), by the [^{14}C] antipyrine method for evidence of metabolic impairment, not only within the developing lesions, but throughout the brain. For this, one monkey was killed at baseline, three during the phase of development of increased CSF pressure, one at its peak, and one upon recovery. Two unirradiated animals served as controls. The findings indicated that as the CSF pressure increased the blood flow decreased. This decrease was at first greater in the right hemisphere, the side of the lesion, and in the white matter more than the grey matter. However at the highest pressures the blood flow was decreased bilaterally in both white and grey matter. Of perhaps grave significance was the only partial recovery in blood flow in the monkey whose intracranial pressure had returned to normal. While there was greater improvement in grey than white matter, there remained a diffuse reduction in blood flow suggesting a long-term impairment in cellular metabolism and/or blood flow regulatory mechanisms.

After these extensive studies of the delayed effects following a single exposure of 3,500 rads of orthovoltage radiation to a limited portion of the brain, we were confronted with two questions: 1) What, if any, of these effects would be observed if the whole brain were exposed to supervoltage irradiation in a single dose, and 2) What, if any, of these effects would be observed if the exposure were divided and delivered to the whole brain in a manner similar to that utilized in standardized therapeutic procedures? Of particular interest was the protocol employed by the Brain Tumor Study Group, sponsored by the Cancer Institute, NIH. For their irradiation of malignant gliomas, they use a 6,000 rads mid plane tumor dose over a period of 6-7 weeks with lateral parallel opposing fields. With megavoltage equipment, source axis distance of not less than 75 cm and a mid plane depth dose rate of not less than 33 rads per minute, daily increments of approximately 200 rads per day are delivered during a five day week.

Current Objective: To determine the effects on normal brain from super voltage radiation within the therapeutic range.

Methods Employed, first: In twelve monkeys, 3 kg in weight, the whole brain was subjected to a single exposure of supervoltage irradiation. Three groups of four monkeys each received 1,000, 1,500 and 2,000 rads, respectively. The source was a linear accelerator with an electron beam energy of 20 MeV, that after collimation was converted to photons by tantalum foils and flattened by a lead disc to provide a nearly constant radiation field. Shielding with three to eight inches of lead provided protection except for an opening limited to the external borders of the brain, as determined by routine skull x-ray. Dosimetry by thermoluminescent dosimeters and ionizing chambers indicated a variation of less than 5% throughout phantom brains, irradiated unilaterally, from the left. The dose rate at mid plane was 200 rads per minute. The source-axis distance was 150 cm. The geometry of the exposure was determined at the Armed Forces Radiobiology Research Institute and the irradiations were carried out in that facility. Four additional monkeys served as controls. When one from each group was killed at 26, 52, 78 or 104 weeks after irradiation the brains were fixed by perfusion with paraformaldehyde, embedded whole in celloidin, and cut in serial sections at a thickness of 35 μ . Every twentieth section was stained for myelin by the Loyez method and the adjacent section for nerve cells and glial nuclei with the Nissl method. An additional set, 100 sections apart, was stained with H & E and PAS for vascular and other structures.

Major Findings: Those with the whole brain exposed to 1,000 rads in a single dose demonstrated no clinical signs. Those irradiated with 1,500 rads showed progressive neurological abnormalities, including diminished motor activity, myoclonic jerks, high amplitude slow waves in the background EEG, papilledema and loss of visual acuity. With whole brain exposure to 2,000 rads, none of the monkeys survived beyond 26 weeks. Their deteriorating condition included crippling loss in motor power and dexterity with finally an inability to ingest food. Three out of the four, had pronounced papilledema beginning in the 8th week, the fourth, had blurred disc margins in the 12th week, that was less evident at the time of sacrifice.

The brains of those exposed to 1,000 rads showed no abnormality. The brains of those subjected to 1,500 rads, that were killed at or beyond 52 weeks showed evidence of swelling and symmetrically dilated ventricular systems. When sectioned, the brain of the monkey killed at 26 weeks, showed scattered focal lesions in the forebrain white matter. These consisted of discrete, minute areas of necrosis, in some of which there were mineral deposits. The monkey killed at 52 weeks, showed wide spread areas of coalescing necrosis involving primarily the white matter of the cerebral hemispheres, along with focal lesions in the brain stem and cerebellum. In the focal lesions there was some mineralization with very little found in the confluent areas of necrosis. Vascular changes included amorphous, subendothelial deposits in the small arteries, and capillary telangiectasis. The monkey killed at 78 weeks, had a pattern of confluent and focal necrosis similar in kind, but less in degree than that shown in the monkey killed at 52 weeks. The monkey killed at 102 weeks, on gross examination showed atrophic as well as oedematous changes, along with cerebellar tonsillar herniation. The histological examination of this brain is

not yet complete. With whole brain exposure to 2,000 rads, the most striking gross abnormality in all four brains was the symmetrical enlargement of the ventricular system. Upon sectioning, they all had a wide scatter of focal necrotic lesions that, though very high in number had not yet shown coalescing or confluence. The large number of fresh lesions in the brain stem undoubtedly contributed to the inability of any of the four to survive beyond 26 weeks. Table 2.

Methods Employed, second: In twelve monkeys, the whole brain was subjected to fractionated exposures of supervoltage irradiation, at approximately 200 rads a day five days a week, with the same equipment described for the previous series. To bracket the human exposure of 6,000 rads, now used for malignant gliomas in twelve centers in the United States, three groups of four monkeys each received 4,000, 6,000 and 8,000 rads in courses of 4, 6, and 8 weeks, respectively.

Major Findings: The monkeys exposed to 4,000 rads showed no functional abnormalities. Those that received 6,000 rads showed alteration in visual evoked response and/or papilledema from the 8th week following irradiation, the latter usually receding between the 16th and 24th week. From the 32nd week high amplitude slow waves were apparent in the EEG. Those that received 8,000 rads exhibited a course not unlike those that received 6,000 rads up to 26 weeks. Beyond that time there was a progressive deterioration reflected in severe impairment in motor function, papilledema followed by optic atrophy and behavioral blindness, and prominent delta activity in the EEG. The brains of the animals that received 4,000 rads showed no structural abnormalities. In those exposed to 6,000 rads, the animal killed at 26 weeks, showed 143 lesions scattered throughout the forebrain, predominantly in the white matter of the corona radiata, centrum semiovale and internal capsule, with a fair number in the central grey matter. These consisted of areas of necrosis, 1 mm or less in diameter, attended with varying degrees of vascular, glial and macrophage reactions. The individual lesions were of different ages. The most recent consisted of a central core of necrotic brain surrounded by a narrow rim of macrophage response. Slightly older lesions contained numerous macrophages throughout. The oldest showed mineral deposits that stained for both calcium and iron. Myelin sections adjacent to the Nissl sections at times revealed corresponding areas of palor that often exceeded in extent the area of necrosis, suggesting focal "vasogenic" edema. Adjacent to the punctate lesions were occasional grossly altered vascular endothelial cells. Quite apart from these, were areas of capillary proliferation, or telangiectasia, 5-8 mm in extent. At 52 weeks there were only 12 small necrotic lesions, and most of these were mineralized. In addition there were innumerable minute areas of mineral deposits that may have represented remnants of larger necrotic lesions, or degenerated vascular walls or plasma products. A more active process was reflected in widespread areas of dilated capillaries or telangiectasia, more than were seen at 26 weeks. At 66 weeks there were 63 small necrotic lesions with varying degrees of associated reactions including mineralization in 49. There were also scattered minute mineral deposits bilaterally. At 78 weeks there was a wide distribution of 242 focal necrotic lesions with a

somewhat greater extension into the brain stem than was seen in the animals that were killed at shorter intervals. The accompanying vascular reactions and mineral deposits were, however, more apparent. From the preceding it appears that in the monkey exposed to 6,000 rads there is a tendency to fewer and fewer necrotic lesions to appear with the passage of time with a progressively larger number that are mineralized, suggesting a tendency toward healing. The monkey killed at 78 weeks showed more lesions than were found at earlier intervals, but few of these were recent, sustaining the impression of a trend toward repair. The effects from 8,000 rads at 26 weeks included 64 focal lesions, that were similar in kind but greater in degree, than those from 6,000 rads. One monkey killed off schedule at 32 weeks, showed 2,089 focal lesions throughout the white and grey matter of the brain, with 115 in the brain stem. At 52 weeks there was a confluence of necrotic lesions in the swollen cerebral hemispheres including thalamus and basal ganglia, but fewer focal lesions in the brain stem than were found in the monkey that was incapacitated at 32 weeks. At 78 weeks there was massive brain destruction from confluent necrotic lesions in the cerebral hemispheres. The loss in brain substance was reflected in the atrophy of the gyri and marked enlargement of the ventricular system. These monkeys exposed to 8,000 rads demonstrate with the passage of time a constant, progressive increase in number and confluence of necrotic lesions. Even within this relentless course the monkey killed at 78 weeks showed a somewhat less active and more atrophic process than the monkey killed at 52 weeks. Table 3.

Present and Future Activities: A definitive series was started in July 1975 that will more closely simulate the human therapeutic experience and provide more precise information regarding the pathogenesis of the radiation lesions. Twenty-one adult male monkeys, 8 to 12 kilos in weight, were selected to more closely approximate in their age the adult humans that are irradiated for malignant gliomas. The brains of these animals were exposed to 6,000 rads of supervoltage radiation in a six week course, with alternate sides of the head exposed on alternate days. They are being killed, in sets of three, at sequential intervals following the beginning of the radiation course, to gain a better concept of the development and degree of recession of the lesions produced by this mode of irradiation. Their clinical course is being monitored by neurological observations, fundoscopic photography and spinal fluid examination for protein and lactic dehydrogenase. To these regularly scheduled examinations there will be added computerized axial tomography, that is expected to be available in May 1976. The latter should not only provide evidence of brain loss as reflected in dilated ventricles but, with the use of enhancement techniques, evidence of breaks in the blood brain barrier that would give a dynamic appraisal of the location and degree of tissue breakdown, and repair.

Significance to Bio-Medical Research and the Program of the Institute: The principal advantage in simulating a therapeutic regime in a monkey model is to observe the effects in a brain uncomplicated by pre-existing pathology, e.g. that resulting from a neoplasm, surgical trauma or chemotherapy. This kind of information will be useful in the planning of therapeutic efforts in man, with attention to "risk-benefit" factors, and in the interpretation of fresh neurological findings following therapy. Further, there is a

direct relevance to a major program of the Cancer Institute, i.e. the Brain Tumor Study Group, and we are privileged to share in their findings as this group is made aware of our results.

Publications:

Caveness, W.F., Kemper, T.L., and Vernon, M.L.: Is an infectious agent involved in the delayed effects of C.N.S. irradiation? Proceedings of the VIIIth Congress of Neuropathology, Budapest, Hungary, September 1-7, 1974, Excerpta Medica, Amsterdam, 1975, pp. 87-95.

Tanaka, A., Ueno, H., Yamashita, Y., and Caveness, W. R.: Regional cerebral blood flow in delayed brain swelling following x-irradiation of the right occipital lobe in the monkey. Brain Res. 96: 233-246, 1975.

Nakagaki, H., Brunhart, G., Kemper, T.L., and Caveness, W.F.: Monkey brain damage from radiation in the therapeutic range. J. Neurosurg. 44:3-11, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 NS 02189-01-LEN</div>
PERIOD COVERED <div style="text-align: center;">July 1, 1975 to June 30, 1976</div>		
TITLE OF PROJECT (80 characters or less) <div style="text-align: center;">Anatomical and Functional Sequelae of Penetrating Head Injury, Phase I</div>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	W. F. Caveness	Chief, Lab. of Exp. Neurology LEN NINCDs
OTHER:	G. H. Weiss S. Jablon B.L. Rish A.M. Meirowsky J. P. Kistler J.P. Mohr V.S. Caviness, Jr.	Chief, Physical Sciences Lab. DCRT Assoc. Director, Med. Follow-up Agency, National Research Council, NAS Assoc. Prof. of Neurosurg., Eastern Virginia Medical School Assoc. Prof. Neurological Surg., Vanderbilt Univ. School of Medicine Instructor in Neurol., Harvard Medical School Assist. Prof. of Neurol., Harvard Medical School Assist. Prof. of Neurol., Harvard Medical School
COOPERATING UNITS (if any) Physical Sciences Laboratory, DCRT, NIH Medical Follow-up Agency, National Research Council, NAS Eastern Virginia, Vanderbilt University and Harvard Medical Schools		
LAB/BRANCH Laboratory of Experimental Neurology		
SECTION		
INSTITUTE AND LOCATION NINCDs, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>Objective: To determine the loss in brain substance and the alteration in brain function, 8-10 years after <u>brain damage</u> incurred during the Vietnam war. The resource for this is a Registry of 1500 Head Injuries compiled in the field by Military Surgeons from 1967 to 1970. The characteristics of the injury and the initial disability is recorded in detail, according to a prospective plan.</p> <p>To achieve this objective, a plan has been developed, titled Phase I, and a team assembled to a) collect, review and analyze the intervening medical records and b) plan, Phase II. The latter will utilize an unique approach to the loss or alteration in structure, i.e., computerized axial tomography, and other techniques, not available in previous studies of sequelae. A Feasibility Study for Phase I will be initiated in the late Spring of 1976.</p> <p>During FY 1976, Project No. Z01 NS 01952-04-LEN was incorporated with this project.</p>		

Project Description

Objectives: To determine the loss in brain substance and the alteration in brain function, 8-10 years after brain damage incurred during the Vietnam War.

Background: A registry for Head and Spinal Cord Injuries, as they occurred in military combat in Vietnam, was developed at the request of the Surgeon General of the U.S. Navy and was implemented with the cooperation of the Surgeons General of the U.S. Army and U.S. Air Force. The purpose was to insure uniformity of data collection and to identify cases for present and future studies. The yield from field surgeons, 1967 to 1970, was 2,043 entries that included 1,683 head injuries, 329 spinal cord injuries and 31 combinations of the two. The average age at time of injury was 21.6 years. A rigorous appraisal demonstrated the uniformity and completeness of 1,540 head injury forms. Ninety per cent of these were the result of explosively propelled missiles. 10% the result of vehicular accidents, falls, or blunt objects. In 85% there was local brain destruction, as evidenced by penetration of the dura with cortical laceration. Sixty per cent of these were accompanied by immediate loss in consciousness, by history. Thirty per cent were in coma responding only to pain at time of examination, that was generally within six hours of injury. This provides an opportunity to group selected cases by regions of focal damage, with and without alteration in consciousness. These categories constitute a background against which the natural history of sequelae may be determined. The analysis of the Registry content was completed in Project No.: Z01 NS 01952-04-LEN.

Current Methods: A contract project entitled, "Anatomical and Functional Sequelae of Penetrating Head Injury, Phase I", was submitted to NINCDS in May 1975. A Feasibility Study for Phase I was approved in March 1976, and will be implemented in May 1976. The conduct of this plan will update the clinical data on 1,500 selected cases from the Registry, by a review of the accumulated Military and Veteran Administration hospital records since the time of injury. The assembly of the records, abstracting the pertinent data, coding, transfer to magnetic tape, and analyses of sequelae, so identified, will be carried out in cooperation with the Medical Follow-up Agency of the National Research Council, NAS, the Physical Sciences Laboratory of the Division of Computer Research and Technology, NIH, and three qualified professionals at the Eastern Virginia Medical School, Vanderbilt University Medical School and Harvard Medical School, respectively. Concomitant with the completion of Phase I will be the planning of Phase II, i.e., an in-hospital examination of three quarters of these head injured casualties with techniques not previously available, including computerized axial tomography for the determination of brain loss. A second project proposal will be required for Phase II.

Significance to Bio-Medical Research and the Program of the Institute: The observations during the acute phase of injury in these cases are more uniform and probably more accurate than any previous series of comparable size. This provides an extraordinary opportunity for studies of prognostic factors and the natural history of disabilities in central nervous system trauma in man. The recent development of new techniques for evaluating

functional deficits, e.g. regarding language and memory and new techniques for determining alterations in brain structure e.g. the CAT Scan, will afford an unique opportunity in Phase II for fresh insight in posttraumatic sequelae and into the function of the remaining brain.

This project is consistent with the efforts to improve the understanding and management of the effects of trauma now being sponsored by the Stroke and Trauma Program of NINCDS and the General Trauma Program of the Institute of General Medical Sciences

The participation of the Medical Follow-up Agency, National Research Council, and the professionals at the Universities indicated are made possible through contract mechanisms.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01952-05-LEN
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RIIOD COVERED July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Vietnam Registry, Analysis of Yield

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PT: William F. Caveness, M.D. Chief, Lab of Exp Neurology LEN NINCDS

OTHER: Berkley L. Rish, CAPT (MC) USNR

COOPERATING UNITS (if any)

LAB/BRANCH Laboratory of Experimental Neurology

SECTION

INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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SUMMARY OF WORK (200 words or less - underline keywords)

During FY 1976 this project was incorporated with Project No. Z01 NS 02189-01-LEN.

ANNUAL REPORT

July 1, 1975 through June 30, 1976

Laboratory of Neurochemistry, Intramural Research
National Institute of Neurological and Communicative
Disorders and Stroke
R. W. Albers, Acting Chief

The projects within LNC are directed toward explaining neural mechanisms in terms of molecular events. These projects reflect the independent and diverse interests of the scientists within the laboratory who have developed a correspondingly wide range of techniques and expertise.

The reports of the individual Sections are summarized below.

The Section on Enzyme Chemistry obtained data on the physical parameters which influence the equilibrium between the two conformations of the ($\text{Na}^+ + \text{K}^+$) ATPase that differ in the orientation of the K^+ ionophor.

New measurements of the rate of phosphorylation of this enzyme which were obtained with high speed quenching apparatus have given indication of the existence of a hitherto unidentified intermediate in the hydrolysis of ATP.

Specific antisera to the polypeptide subunits of the ($\text{Na}^+ + \text{K}^+$)-ATPase have been employed in studies of the localization of this enzyme within the central nervous system and also to study the organization of the enzyme subunits within the plasma membrane.

The Section on Physiology and Metabolism has extended its studies on ecto-enzymes in cultured cell lines to a study of a Ca^{++} -stimulated ATPase in different strains of mice that vary in susceptibility to audiogenic seizures. The more susceptible mice exhibit lower Ca^{++} -ATPase activity. From studies of the property of this enzyme as a function of age, it was inferred that several different isozymes may appear at different stages of brain maturation.

This section also maintains an interest in the use of marine organisms in the study of membrane processes. Methods have been developed for isolating plasma membranes from sea urchin embryos, preparatory to a study of plasma membrane biosynthesis.

Studies of the Section on Neural Development and Regeneration are directed to problems of nerve regeneration and the determinants of functional neuronal connections. Ganglion transplants are being studied with respect to the immunological factors that determine survival or rejection and permit successful innervation of end-organs.

Functional homografts of adult ganglia, by these criteria, have occurred between animals that differ in both major and minor histocompatibility loci and that have been made immunologically tolerant.

Homografts between animals that differ only in the minor histocompatibility loci have survived 35 days without induction of immune tolerance, although rejection ultimately occurred.

Studies of the neurotrophic factor that induces tongue epithelia to differentiate into taste buds are underway utilizing the techniques developed in the transplant studies.

The Section on Amino Acids and Electrolytes is engaged in a study of regulation of Na^+ and K^+ balance in brain tissue of hibernators. The primary interest has been to elucidate the factors that permit Na^+ and K^+ active transport to operate with increased efficiency at low temperature. This appears to result from changes in the lipid composition of plasma membranes. Measurements of the microviscosity of cell membranes and their lipids were

obtained comparing preparations from hibernating and from warm-adapted hamster brains. The results substantiate the importance of changes in membrane lipids in determining the physical properties of the membrane. Comparative studies of the kinetics of the $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ demonstrate certain stages of the mechanism that are particularly sensitive and play a role in adaptation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do not use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 00813-15 LNC									
PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p>											
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Enzymological Aspects of Neural Function</p>											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 60%;">R. W. Albers, Section Chief, Lab. of Neurochem.</td> <td style="width: 25%;">LNC NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>D. H. Jean, Visiting Associate</td> <td>LNC NINCDS</td> </tr> <tr> <td></td> <td>L. Rodichok, Research Associate</td> <td>LNC NINCDS</td> </tr> </table>			PI:	R. W. Albers, Section Chief, Lab. of Neurochem.	LNC NINCDS	OTHER:	D. H. Jean, Visiting Associate	LNC NINCDS		L. Rodichok, Research Associate	LNC NINCDS
PI:	R. W. Albers, Section Chief, Lab. of Neurochem.	LNC NINCDS									
OTHER:	D. H. Jean, Visiting Associate	LNC NINCDS									
	L. Rodichok, Research Associate	LNC NINCDS									
COOPERATING UNITS (if any) <p style="text-align: center;">J. Froehlich, Laboratory of Molecular Aging, NIA, NIH, Bethesda, Maryland.</p>											
LAB/BRANCH <p style="text-align: center;">Laboratory of Neurochemistry</p>											
SECTION <p style="text-align: center;">Enzyme Chemistry</p>											
INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</p>											
TOTAL MANYEARS: <p style="text-align: center;">4.5</p>	PROFESSIONAL: <p style="text-align: center;">2.5</p>	OTHER: <p style="text-align: center;">2.0</p>									
SUMMARY OF WORK (200 words or less - underline keywords) <p>Current studies are directed toward elucidation of the mechanism and structure of the $(Na^+ + K^+)$-ATPase which is the enzymatic expression of active sodium transport. The two polypeptide subunits have been isolated from the enzyme. Antisera to each have been prepared and employed in studies which indicate that the two antigenic loci are well separated.</p> <p><u>Transient kinetics</u> of phosphorylation show evidence for a new enzyme intermediate which accumulates in the early stages of the ATPase reaction.</p> <p>Steady-state kinetics have shown a pH and temperature dependence of the <u>conformational equilibrium</u> of the ATPase.</p>											

Project Description:

Objectives: To assess the functions, molecular structures and mechanisms of enzymes and enzyme systems which characterize neural tissues or which respond to neural influences. Current studies are directed toward elucidation of the mechanism and structure of the $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ which is the enzymatic expression of active Na^+ transport and hence of the membrane potential-generating system of neural tissues.

More specific objectives of the current year have been: (1) To investigate the functional and structural relationships between the two different polypeptide subunits of the $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$; (2) To obtain information about the mechanism of the transport process through the measurement of the transient kinetics of enzyme phosphorylation and hydrolysis.

Methods: Isolated plasma membrane fragments from brain and *Electrophorus* electric organ are the starting materials for our $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ studies.

The further purification of the enzyme depends upon dissociation of the membranes with detergent. The detergent-solubilized enzyme can be isolated from other membrane proteins by preparative gel electrophoresis. The subunits are dissociated by sodium dodecyl sulfate and isolated by preparative gel electrophoresis.

Standard techniques for producing anti-sera in rabbits are employed using these proteins as antigens.

The rapid kinetics of phosphorylation and hydrolysis employ an apparatus designed by Dr. Froehlich of NIA which permits quenching of the enzymic reaction with acid with a time resolution of 3 milliseconds.

Steady-state kinetics employing spectrophotometric measurement of initial enzyme rates have been applied to a study of the pH and temperature effects on the conformation of the enzyme.

Major Findings: (a) Our earlier studies have defined two distinct conformations of the $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ which differ in their binding constants for K^+ . We have now shown that the equilibrium between these two conformations is sensitive to temperature and to pH. The form with high K^+ -affinity is favored by low temperature and low pH. The tentative conclusion is that these two forms differ in the orientation of the K^+ ionophor with respect to the intra- and extracellular fluid phases.

(b) The specific antisera to the detergent-solubilized ATPase and to the individual ATPase subunits will bind to the ATPase-containing membrane fragments. We have shown that the antibody to each subunit specifically protects that subunit from proteolytic degradation by trypsin. Binding of an antibody to one subunit does not prevent binding of antibody to the other subunit despite the large size of the antibody molecule. Taken together with other evidence, this suggests that the antigenic sites for

the two subunits reside on opposite sides of the plasma membrane.

(c) Transient kinetics of the enzyme phosphorylation and subsequent hydrolysis have been measured at different ATP concentrations. We have established that the phosphoryl enzyme is formed earlier than the first appearance of inorganic phosphate. This is firm evidence that the phosphoryl enzyme is a normal intermediate of the reaction. Moreover, the initial level of phosphoryl enzyme may exceed its steady-state level. This indicates the existence of a subsequent rate-limiting intermediate, hitherto undetected. A reaction sequence which includes a slowly-dissociating, acid labile phosphate intermediate can be fit to the kinetic data. Computer simulation has permitted calculation of rate constants for each step of the mechanism.

Proposed Course: The transient kinetic studies give some support to the proposal that the ATPase may exhibit the "half-of-sites" phenomenon. To act in this way an enzyme must be organized in dimeric form, each monomer participating in the catalytic cycle 180 degrees out of phase with the other. Experiments are planned to test this possibility.

Several lines of evidence suggest that the organization of the sodium pump ATPase in membranes is subject to various control mechanisms. These controls may involve a more highly organized complex of protein molecules than is evident in the detergent-solubilized enzyme. It is proposed to explore methods which may permit elucidation of the topographical and functional organization of proteins within the membrane. We have begun some exploration of the use of antibody localization of ATPase at the electron microscopic level of resolution. This work appears to confirm the biochemical evidence that the major antigens of the two subunits are on opposite sides of the membrane. Information about the disposition of subunits within the plane of the membrane will require some improvement in resolution. A preliminary collaboration, with Drs. John Wood and Thomas Reese, indicates that the surface replica technique may be valuable for these studies. We intend to continue the development of the use of immunochemical techniques for the localization of membrane proteins.

Preliminary studies are underway to characterize the regulation of guanine nucleotide metabolism in brain. GTP is specifically required for such functions as microtubule assembly, protein glycosylation and adenylyclase activation, as well as 3',5'-cGMP synthesis. GTP is produced at one point in the Krebs cycle and may be regulated separately from ATP production although there appear to have been no studies directed to this question. Depending upon the outcome of these studies, a new project may be initiated.

Significance: Active Na^+ transport appears to be the principal metabolic activity of nerve cells. In most cases it is coupled to the accumulation of K^+ . Extrusion of intracellular Na^+ is the basis for maintaining the resting membrane potential of nearly all animal cells. In the nervous system, it provides the linkage through which oxidative metabolism supplies energy to

produce the nerve action potential, to release neurotransmitters, and to reaccumulate these transmitters or their metabolites.

With respect to the more general aspects of cell metabolism, Na^+ transport is closely linked to cellular uptake of carbohydrates, amino acids and other nutrients.

Various investigators have proposed that malfunctions of this system may be involved in such pathologies as epilepsy, myotonias, muscle atrophy, slow virus encephalopathy, etc.

Publications:

1. Albers, R. W.: The (Sodium plus Potassium)-Transport ATPase in "The Enzymes of Biological Membranes," Vol. 3, pp. 283-301, edited by A. N. Martonosi, Plenum Press, 1976.
2. Froehlich, J. P., Albers, R. W., Koval, G. J., Goebel, R. and Bergman, R.: Evidence for a New Intermediate State in the Mechanism of $(\text{Na}^+ + \text{K}^+)$ -Adenosine Triphosphatase. J. Biol. Chem., in press, 1976.
3. Jean, D. H., Albers, R. W., Guth, L. and Aron, H. J.: Differences between the Heavy Chains of Fast and Slow Muscle Myosin. Experimental Neurology 49: 750-757, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01480-09 LNC

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Metabolism of Neurohumoral Transmitter Substances in Marine Animals

NAMES, LABORATORY AND INSTITUTION AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: E. G. Trams Chief, Sect. Physiol. & Met. LNC NINCDS

COOPERATING UNITS (if any) A. A. Benson, Assoc. Dir., Scripps Inst. Ocean., LaJolla, CA
S. Patton, Borland Prof. Food Sci., Penn. State Univ. Park, PA
E. A. Brown, Pharmacologist, Pulmonary Branch, NHLI
P. Gilbert, Director, Mote Marine Laboratory, Sarasota, FL

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Physiol. and Metabolism

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to explore the great variety and abundance of the marine environment for molecular models of neurochemical systems. In particular we search for species or phenomena which display an amplification (or simplification) of human or mammalian physiology (e.g. electric eel, aplysial). We have used the sea urchin embryo to study the biosynthesis of plasma membranes. Of special interest to us is the question how certain membrane constituents are built into the membrane or area-activated into functional units there. We have also tried this year to obtain primary cultures from the brains of teleosts and elasmobranchi.

Project Description:

Objectives: To exploit for the study of neurobiology the great variety and abundance and the biochemical specialization of oceanic life. In particular we have selected to study some aspects of neurochemistry in marine organisms where certain tissues, species or phenomena constitute an evolutionary amplification of normal human physiology.

Methods: Our studies have been concerned with the metabolism of plasma membranes and transmitter substances. The methodology in use is adjusted to specific requirements according to current laboratory techniques. Use is made of radioactive precursors, i.e. labeled biogenic amines are introduced for studies of their metabolism in vivo or in vitro. Common enzymological approaches are used when appropriate, especially in drug metabolism, toxicology, and when the effects of putative transmitters on metabolic pathways are investigated.

Experimental animals or organisms used: various kinds of Selachians, Torpedinidae, several species of Teleosts, in particular Electrophorus electricus and Pacific salmon (*Oncorhynchus* spp.); small crustaceans, Ecchinoderms, Aplysia. Emphasis has been placed on the study of membranes, electric organs and nervous tissues or tissues which are of interest to our principal program on structure and function of excitable membranes.

Major Findings: This past year we have made a first attempt to culture CNS derived cells from teleosts and selachians. We have used essentially the same techniques which we employ for the culture of primary glia cell lines from neonatal mice. This work was done at the Mote Marine Laboratory with the collaboration of the staff there. We have not yet succeeded in establishing a viable cell line from fish brains and we will repeat this attempt under improved conditions.

We have also continued our studies on the plasma membranes of developing sea urchin embryos. The sea urchin provides us with an elegant tool for the study of plasma membrane biosynthesis. Following fertilization the egg divides approximately every hour and during the first ten divisions about one thousand cells are formed. The plasma membrane during the same period increases about ten-fold while biomass of the embryo does not change. Our main interest in recent investigations has been focused on enzymes which occur characteristically in the plasma membrane. We have explored a variety of techniques to prepare plasma membranes from developing sea urchin embryos. One of the difficulties in making well controlled studies on plasma membrane biosynthesis in sea urchins is that, as the embryo develops through its initial stages, the density of the embryo and of the plasma membrane changes. Therefore the conditions for isolating membranes from a 4 cell stage embryo are different from those used for a 64 or 1024 cell embryo.

We now have the methods fairly well worked out for preparing partially purified plasma membranes from the urchin embryo.

Proposed Course: On partially purified plasma membrane preparations we will study the characteristics of several phosphoesterhydrolases. The proposed investigations interdigitate with other work currently going on in our laboratory and which will be described in detail in Project No. Z01 NS 01481-09 LNC.

Publications:

1. S. Patton, I. M. Zulak and E. G. Trams: Fatty acid Metabolism via Triglyceride in the salmon heart. J. Mol. Cell. Cardiol. 7, 857-865, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT: JABER (Do not fill in this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01481-09 LNC												
PERIOD COVERED July 1, 1975 to June 30, 1976														
TITLE OF PROJECT (80 characters or less) Studies on the composition and metabolism of cellular membranes														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0"> <tr> <td>PI:</td> <td>E. G. Trams, Chief, Section Phys. & Metab.</td> <td>LNC NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>C. J. Lauter, Chemist</td> <td>LNC NINCDS</td> </tr> <tr> <td></td> <td>W. Reichert, Staff Fellow</td> <td>LNC NINCDS</td> </tr> <tr> <td></td> <td>D. E. Rosenblatt, Staff Fellow</td> <td>LNC NINCDS</td> </tr> </table>			PI:	E. G. Trams, Chief, Section Phys. & Metab.	LNC NINCDS	OTHER:	C. J. Lauter, Chemist	LNC NINCDS		W. Reichert, Staff Fellow	LNC NINCDS		D. E. Rosenblatt, Staff Fellow	LNC NINCDS
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COOPERATING UNITS (if any) None														
LAB/BRANCH Laboratory of Neurochemistry														
SECTION Physiology and Metabolism														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014														
TOTAL MANYEARS: 2.8	PROFESSIONAL: 1.8	OTHER: 1.0												
SUMMARY OF WORK (200 words or less - underline keywords) The objective of this project is to elucidate the inter-relationship between molecular composition of the <u>plasma membrane</u> and the functions that the membrane building blocks serve in <u>transport</u> , <u>bioelectrogenesis</u> and <u>inter-cellular communication</u> . Plasma membranes are isolated from tissues (brain) or from <u>cultures</u> of CNS derived cells and membrane-characteristic <u>macromolecules</u> are studied, isolated and purified. In particular the function of <u>membrane ecto-enzymes</u> was one of the objectives of our studies. Some of this work may have a possible bearing on the biochemistry of CNS cells in <u>convulsive diseases</u> . We have found that in <u>seizure-prone mice (DBA)</u> there is a deficiency of a <u>Calcium-activated ATPase</u> . This ATPase is probably identical with one of the ecto-enzymes, which we identified in glia or neuroblastoma. Other animal models of epilepsy are studied.														

Project Description:

Objectives: To elucidate the inter-relationship between the molecular composition of the plasma membrane and the functions that macromolecular building blocks serve in transport, bioelectrogenesis and intercellular communication.

Methods: We continued the propagation of various tissue culture cell lines in the laboratory. Established clones of mouse neuroblastoma (N-18) human astrocytoma (Cox Clone), rat glioma and neonatal hamster (NN) astrocytes were carried in routine monolayer cultures. In addition, we have developed cell cultures from neonatal mouse brain. These monolayer cultures have served for the study of ecto-enzymes of the plasma membrane. We also have used the cell cultures as sources for plasma membrane preparations, as enzyme sources and for a variety of metabolic studies concerned with the role of adenine nucleotides as modulators of cell permeability. We have applied our previous findings on the roles of extra-cellular ATP and the ecto-enzymes to investigations of their possible role in convulsive disorders. We have measured a variety of plasma membrane ecto-enzymes in brains of mice which are susceptible to audiogenic seizures. In addition we have investigated the effects of sialidase treatment on ecto-enzyme activity.

Major Findings: It is possible that human epilepsy is caused by qualitative or quantitative molecular incompetencies of brain cells. With certain animal models of the disease the assumption may be readily conceded, because some genotypes will produce seizure-prone individuals with consistency. The analogy to molecular diseases where a lack of enzymes produces metabolic disorder is compelling. Our working hypothesis was based on observations of two phenomena which may have an intimate physiological relationship. 1) We had previously found that several different cell types display ecto-enzyme activities, among which a plasma membrane ecto-ATPase (stimulated by Ca^{2+} or Mg^{2+}) was most prominent. It can now be implied from reports of other investigators that ecto-enzymes are common and perhaps ubiquitous in eukaryotic cells. 2) We have observed that the addition of ATP to the medium of certain cultured cell lines produced a transient increase in membrane permeability. Astrocyte cultures derived from brains of neonatal Syrian hamsters (NN) responded to ATP concentrations as low as 10^{-6}M . Similar effects of ATP have been reported by others in various systems.

In an organized cell system, such as the brain, the translocation of cytoplasmic ATP to the cell surface may have an effect equivalent to the addition of exogenous ATP to cell cultures. If so, ATP may play a physiological role and function to effect transient membrane destabilization.

If cytoplasmic ATP were to react with the cell surface, triggering an increase in permeability and efflux of K^+ into the extracellular space, a lowering of excitability threshold might ensue. One of the functions of plasma membrane ecto-ATPase might be to terminate, or moderate, this effect

of ATP. We argued that an impairment of ecto-ATPase function would result in a de-stabilization of the controls critical to the maintenance of the excitability threshold. Susceptibility to convulsive seizure could thus be related to a qualitative or quantitative incompetency of membrane ecto-ATPase. In brain homogenates, the total Ca^{2+} -stimulated ATPase activity does not only reflect ecto-ATPase activity, but a part of the Ca-ATPase in homogenates showed the characteristics of ecto-ATPases observed in cell cultures derived from the CNS. In order to obtain some measure of the activity of other characteristic plasma membrane enzymes we have assayed the homogenates for 5'-nucleotidase and p-nitrophenylphosphatase.

It was also known that in rats older than 18 days, visual deprivation lowers electroshock seizure threshold and decreases brain Mg^{2+} -ATPase and $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$. We investigated changes of cerebral Mg^{2+} -ATPase and $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ in the audiogenic seizure model of auditory deprivation. DBA/2N and C57B1/6N strains were chosen for their differences in seizure susceptibility. For the $\text{Na}^+, \text{K}^+\text{-ATPase}$ experiments, a total of 28 litters (13 DBA/2N, 15 C57B1/6N) and 150 animals (71 DBA/2N, 79 C57B1/6N) were used. Using nonexposed littermates as controls, mice were individually exposed to a 110 db re 0.002 dyne/cm². We were unable to detect differences in cerebral Mg^{2+} -ATPase and $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ activities with sound exposure and audiogenic seizures. Both seizure susceptible and seizure resistant strains were investigated and animals were examined at ages when they were susceptible to the convulsive effects of sound. Enzyme activities were assayed in homogenate, microsome and synaptosome fractions. Although almost 90% of the sound treated DBA/2N animals had a generalized seizure, enzyme activities were not significantly different from control values.

There were many similarities between the Ca^{2+} -stimulated ATPase in mouse brain homogenates and ecto-ATPases observed in monolayer cultures of mouse neuroblastoma (N-18), neonatal astrocytes (NN) and human astrocytoma (Cox Clone). In tissue cultures, optimal Ca^{2+} concentrations were between 0.1 and 0.2 mM while in mouse brain homogenates 1-2 mM Ca^{2+} was required for optimal activity. The Ca^{2+} -ATPase was not inhibited by ouabain, N-ethylmaleimide, colchidine, or barbiturates in either brain homogenates or tissue cultures. No stimulation of activity was obtained by the addition of dithiothreitol or p-mercaptoethanol. In mouse brain and in tissue cultures, the enzyme was inhibited 20-30% by equimolar concentrations of ADP or 5'-adenylylmido-diphosphate (AMP-PNP). Neither adenosine nor 5'-AMP was inhibitory.

There were consistent differences between the activity of Ca^{2+} -ATPases in the DBA and control (C57 & C3H) mice throughout the entire observation period. The enzyme deficiency in the DBA enzyme assayed about 30% less than the C57 or C3H enzymes. Thereafter the Ca^{2+} -ATPase in DBA was constant at 25% below that of C57 or C3H. The deficiency of the enzyme in the DBA mice was more obvious in the experiments in which the kinetic constants were estimated. There was a marked difference in the V_{max} of the Ca^{2+} -ATPase in the two populations and a substantial increase in rate was evident during

the 21-28 day period. The apparent K_m in both populations more than doubled between 7 and 60 days. Furthermore, the K_m values which we obtained seemed to fall into sets grouped around the values of 0.17, 0.23, 0.33 and 0.42 mM for ATP.

Upon assay of Ca^{2+} -stimulated ATPase in F_1 hybrids of DBA x C57, we found that the activity was about half-way between those of the parent strains. One explanation for the co-existence (or sequential predominance) of different Ca^{2+} -ATPase isozymes is that a pattern based on molecular phylogeny is repeated as the animal ages.

5'Nucleotidase is probably the most widely accepted plasma membrane marker enzyme, though it is not necessarily a component which is unique to plasma membranes. p-Nitrophenylphosphatase occurs in several forms; the so-called neutral p-NPPase, which was determined in these experiments, is thought to be a constituent element of the sodium transport system (Na, K^{+} -ATPase) and therefore localized predominantly in the plasma membrane. The activities of these two enzymes at each time period were virtually the same in DBA, C57 and C3H mice. Both enzymes showed a substantial increase in specific activity in our brain homogenate preparations between day 21 and 28. If they are viewed, however, as an index of plasma membrane function it can be concluded that no generalized impairment of the membrane existed in the DBA mouse.

The deficiency in the Ca^{2+} -ATPase which we report here appears to be a rather moderate one, especially in comparison to some other molecular diseases where pronounced enzyme deficiencies occur. Our enzyme preparation was not expected, however, to differentiate between several Ca^{2+} -stimulated ATPases which have been found in brain. We were also aware of the limitations imposed by the use of animal models for epilepsy. The use of DBA mice in particular has been reviewed by several investigators. Other models may be equally pertinent or misleading. Conceivably, individuals are convulsors when the frequency of genes conditioning susceptibility passes a threshold. Applied to our concept of an underlying error in ecto-ATPase performance, this can be restated as a lack of genes for the synthesis of a Ca^{2+} -ATPase isozyme during a specified period. Susceptibility is manifested when a lower enzymic threshold is passed. Enzyme polymorphism or different gene expressions for the same catalytic activity has been widely accepted in a variety of disorders. We interpret the data from the Ca^{2+} -ATPase kinetics experiments as representing a pattern of sequential production of isozymes. Differences between DBA and control animals appear just prior to the period of maximum seizure sensitivity. These differences may reflect failure to either synthesize a Ca^{2+} -ATPase isozyme or delay of its synthesis. This enzymatic defect, for reasons which were stated in our working hypothesis, may render the animal temporarily sensitive.

Proposed Course: We shall make further investigations into the role of the ecto-enzymes. Although our attention is focused on experiments which relate to inter-cellular communication and excitability, we shall keep in mind that the ecto-enzymes may serve other phenomena, such as cell adhesion or recognition or in micropinocytosis.

We are also planning to use other animal models for the study of a possible molecular deficiency in convulsive diseases.

Publications:

1. Reichert, W. H.: Cerebral Magnesium and Sodium-Potassium ATPase following audiogenic seizures in mice. Exp. Neurol. 49: 596-600 (1975).
2. Trams, E. G. and Lauter, C. J.: Adenosine deaminase of cultured brain cells. Biochem. J. 152: 681-687 (1975).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01586-09 LNC
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) Regeneration and trophic function of neurons in the peripheral nervous system		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: A. A. Zalewski Sec. Head, Nerve Regeneration LNC NINCDS		
COOPERATING UNITS (if any) W. K. Silvers, Dept. Human Genetics, University of Pennsylvania H. G. Goshgarian, Guest Worker, Post Doctoral from Paralyzed Veterans of America		
LAB/BRANCH Laboratory of Neurochemistry		
SECTION Section on Nerve Development & Regeneration		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to investigate the mechanisms by which injured neurons regenerate their nerve fibers and to evaluate neuronal and extra-neuronal factors that may influence the establishment of functional connections. Presently <u>neuronal transplantation</u> studies are being performed to determine whether neurons in <u>homografts</u> survive or are rejected by the <u>immune response</u> and whether neurons if they survive (<u>immunosuppression</u> may be needed to insure survival) can regenerate their nerve fibers and innervate appropriate end-organs. The results to date show that neurons can survive in homografts of ganglia if the recipient animal (rat) is rendered <u>immunologically tolerant</u> . Furthermore these neurons seem capable of surviving permanently (18 months to date) in the tolerant host and they can regenerate nerve fibers into tongue tissue and still perform their <u>trophic function</u> of inducing <u>taste buds</u> .		

Project Description:

Background of Project: The purpose of this project is to investigate the mechanisms by which injured neurons regenerate their nerve fibers and to evaluate neuronal and extra-neuronal factors that may influence the establishment of functional connections. The interaction occurring between neurons and taste buds is being used as a model since taste buds develop under the influence of the nerve, the nerve is required for the maintenance of the buds, and because the buds disappear after denervation and reappear after nerve fiber regeneration. Some insight into the factors responsible for taste bud formation might be obtained if a tissue recombination technique were used to determine any neuronal and tissue (i.e., epithelial) specificities involved in bud formation. Farbman (J. Cell Biol. 52: 449, 1972) has tried tissue culture techniques but he as well as I have not been successful in producing any consistent growth of buds. On the other hand, I have found many regenerated taste buds when a sensory ganglion (i.e., vagal node) and a tongue graft (i.e., containing the vallate papilla) were transplanted and combined in the anterior chamber of the eyes of rats. These initial studies were performed with tissue isografts (grafts between genetically similar animals which do not evoke an immune response) but recently we have repeated them and combined homografts of ganglia (grafts between genetically different animals of the same species which do evoke an immune response) with tongue isografts. We used homografts of ganglia in order to determine (1) whether neurons would survive or be rejected by the immune response and (2) whether homografted neurons would regenerate their nerve fibers and form functional connections. Before describing the results obtained it is pertinent to review the factors which constitute the basis of homograft rejection:

- (1) the antigens which elicit the immune response which results in homograft destruction are called histocompatibility antigens;
- (2) the development of these antigens is under genetic control and the genes concerned are called histocompatibility genes;
- (3) a number of different genetic loci, which are located on different chromosomes, code for histocompatibility antigens;
- (4) histocompatibility genes are codominant, that is both genes on homologous chromosomes express their product and if two different genes are present two different histocompatibility antigens are produced (this differs from the more common dominant--recessive gene traits where a dominant gene may prevent any expression of the recessive gene product);
- (5) all histocompatibility antigens are not of equivalent potency in regard to the intensity of the immune response they provoke;

- (6) one histocompatibility locus codes the most potent antigens and homografts containing them are rejected rapidly. (This locus is referred to as the major histocompatibility locus, named H-2 in the mouse, H-1 or Ag-B in the rat, and HL-A in man, and its antigens are designated as major histocompatibility antigens);
- (7) all other histocompatibility loci specify weaker antigens and trigger milder immune responses so that homografts containing them experience extended survival;
- (8) randomly bred individuals are genetically different and as a consequence these homografts will usually have different major and minor histocompatibility antigens;
- (9) it is possible to have identical genes at the major locus (and therefore the same major histocompatibility antigens) because this locus segregates as a single unit and there is little gene recombination (e.g., two children may have the same major genes but would differ at minor ones since minor ones are multiple, have frequent gene crossing-over, and are inherited independently);
- (10) a given homograft may have different major and minor histocompatibility antigens or only different minor ones relative to the recipient;
- (11) the type of major antigens on a homograft can be identified (tissue-typing) by performing an appropriate serological study and a lymphocyte stimulation test (minor antigens cannot as yet be typed).

In view of the above, homograft studies were performed with Lewis (LE), Fischer (FR), and Brown Norway (BN) inbred rats. The LE and FR rat strains are identical at the major locus but differ at minor ones so that homografts between them assess the immune response to minor histocompatibility antigens. On the other hand, BN rats differ from the LE and FR rats at the major and minor loci and grafts between LE and BN or FR and BN animals evaluate the immune response to major and minor histocompatibility antigens. This protocol offers information as to what might happen in clinical transplantation since the LE and FR results could be related to sibling transplantation when major compatibility exists and only minor histoincompatibilities prevail and the LE and BN findings related to homografts from random donors wherein major and minor histocompatibility differences occur.

Pertinent Past Studies: Previous studies demonstrated that neurons in homografts of ganglia were rejected within 35 days when LE ganglia were transplanted into the anterior chamber of the eyes or into the sternomastoid muscles of BN rats. However, if BN rats were rendered immunologically tolerant, LE neurons survived and regenerated nerve fibers into BN tongue grafts and caused the regeneration of taste buds. Two important questions

to be answered are (1) how long can LE neurons survive in tolerant BN rats, (2) are long-term surviving LE neurons functional (i.e., can they induce taste buds). The solution to the latter question posed a problem since tongue grafts develop cysts after 50-70 days in the eye. Encystment caused the tongue epithelium to become atrophied and usually few or no bud developed. Three ways were considered for determining the long-term functional capacity of homografted neurons in tolerant rats: (1) transplant the ganglion, wait the desired length of time, and then add the tongue graft; (2) devise a new method of putting the tongue graft in the eye that would prevent cyst formation; and (3) reinnervate the tongue "in situ."

In contrast to the results with LE and BN animals, neurons survived at 35 days when FR ganglion were transplanted to LE recipients. The ultimate fate of these homografts, which bear only minor histocompatibility antigens, needs to be determined, and if rejection occurs, experiments to induce immunological tolerance and to show functional regeneration must be undertaken.

Methods Employed: BN rats were made immunologically tolerant of LE grafts by injecting the BN rats as neonates with (LE x BN)_{F₁} bone marrow cells. The immune system of the BN rat is not fully competent at birth and as a result of the exposure to LE histocompatibility antigens it develops a tolerance to LE antigens and subsequent LE grafts survive. When the neonatally-treated BN rats were 100 days old they received a neonatal LE ganglion into each eye and each sternomastoid muscle. A BN tongue graft was combined with the LE ganglion in one eye so that the functional capacity of homografted LE neurons could be determined. The LE ganglion and tongue graft in the eye and one LE ganglion in muscle were examined at 70 days. The LE ganglion in the other eye and muscle were used to determine the long-term survival and function of the LE neurons in tolerant (determined by the findings in the 70 day LE grafts) BN rats. Eleven tolerant-BN rats survived 18 months after receiving the LE ganglia and at this time a BN tongue graft was placed into the eye in contact with the purportedly surviving LE ganglion. We were confident LE neurons were present at 18 months because we examined LE ganglia from other tolerant BN rats at regular intervals and always found neurons. Furthermore, we could by visual inspection follow the size of the eye graft and its constant size and appearance indicated neurons probably were present. While the above experiment was in progress a new technique of putting the tongue graft in the eye was devised. Instead of putting it in the anterior chamber of the eye, the tongue graft was exteriorized by placing it in the confines of a corneal biopsy opening. Thus the epithelial surface of the tongue now faced outward and was not covered by the cornea. Some tongue grafts were exteriorized in the eye without adding a ganglion while in other eyes a ganglion was first put in and then the tongue graft added and exteriorized. Since this was a new technique most grafts were performed with isogenic grafts and not until the merit of the "exteriorized technique" proven were tolerant animals used. The exteriorized grafts were examined after 90 days, a time at which anterior chamber tongue grafts would have developed into cysts. The fate of

neurons in adult homografts of ganglion between LE and FR rats (i.e., grafts with only minor histocompatibility differences) was determined by placing ganglia in the eyes or muscles. In anticipation of rejection some LE or FR neonates were injected with appropriate lymph node cells to induce tolerance to LE or FR homografts. In this experiment grafts and tolerance were studied in reciprocal homografts in both the LE and FR animals. Also we were comparing the effectiveness of lymph node cells to bone marrow cells in inducing tolerance. Tongue grafts were added to the tolerant LE or FR animal to test the functional capacity of surviving neurons. In all studies normal rats were used to determine if rejection of homografts did occur. Frozen sections of ganglia were examined by light microscopical histochemical stains in order to determine the presence of neurons, nerve fibers, taste buds, and immune cells.

Major Findings:

1. Normal BN rats rejected neonatal LE ganglia with the result that no neurons were present in them. It did take 70 days for rejection to occur which is twice the time it takes to reject adult LE ganglia. LE neurons did survive in homografts in tolerant BN rats at 70 days and they were able to induce the regeneration of taste buds. In fact LE neurons survived in tolerant BN rats at all times LE ganglia were examined (i.e., at 6, 8, 10, 14, and 18 months). When tongue isografts were added to the LE ganglia at 18 months, 8 of 11 developed taste buds. Thus, after being isolated for 18 months, LE neurons could if presented with an appropriate end organ regenerate their nerve fibers and cause bud regeneration. It is noteworthy that there was no significant difference in the number of surviving LE neurons over the 18 months' period that they were in the eye or muscle of tolerant BN rats.

2. The exteriorized tongue grafts in isogenic or tolerant animals did not develop cysts and their morphology was well preserved at 90 days. It is very probable that the exteriorized graft would be maintained indefinitely. It is of interest (1) that the exteriorized tongue graft was preserved regardless of whether a ganglion was added or whether the tongue graft was alone; (2) that taste buds were found in the ducts of von Ebner's salivary gland; (3) that nerve fibers intrinsic to the eye induced buds (i.e., buds appeared in tongue grafts to which no ganglion was added); and (4) that two types of nerve fibers (based on their cholinesterase staining) caused taste bud regeneration. Examination of taste buds in their normal locations also revealed two different types of nerves present (a previously unreported finding) thus confirming the eye results.

(3) Neurons in homografts from FR to LE or from LE to FR rats were ultimately rejected. The speed of rejection was variable and some grafts were not rejected until 100 days. Previously all homografts with minor antigens had neurons at 30-50 days. Tolerance was successfully induced in both rat strains by neonatal-lymph node treatment. Both tolerant strains had similar numbers of neurons in homografts and these numbers compared favorably to those found in tolerant animals treated with bone marrow. Examination of

tongue grafts combined with these ganglia showed that some buds appeared. However, the tongue grafts had formed cysts and this probably explains the low yield of buds. Exteriorized grafts combined with these ganglia probably would yield more buds. Currently we have examined LE to FR or FR to LE ganglia in tolerant recipients up to 6 months and found surviving homografted neurons.

Significance:

1. The present results indicate that neurons in homografts which differ in major and minor histocompatibility antigens are acutely rejected whereas neurons differing in minor antigens enjoy longer survival but ultimately they are rejected. However, tolerance to all types of homografts can be induced with the result that neurons will survive and function permanently. In contrast to other tissue homografts like skin where only bone marrow induced tolerance, tolerance to neuronal homografts was induced effectively by bone marrow or lymph node cells. The finding that neonatal neurons could survive in homografts indicates that central nervous system homografting can be performed. Previous attempts to transplant adult CNS tissues failed because no neurons survived beyond the first few days of transplantation. However, it is now known that neurons in neonatal or embryonic CNS isografts can survive, even in situ for up to 12 months (Das, T.I.T., J. Life Sci., 4: 93, 1974). This result coupled with our data that neonatal neurons in homografts can survive permanently in animals rendered tolerant by adult bone marrow or lymph node cells makes it feasible to study the possibility that CNS homografts can be used to replace or promote the repair of injured CNS tissue. The recent report that mucolytic and proteolytic enzymes promote the structural and functional restitution of CNS nerve fibers (Matinian and Windle, Anat. Rec., 181: 423, 1975) encourages the exploration of therapeutic uses of neuronal grafts.
2. The finding that exteriorized tongue grafts survive and maintain their morphology for prolonged periods makes several lines of research possible. For example, it is possible to create a barrier between ganglia and tongue graft to determine whether physical contact is needed for nerve to induce the buds or whether a freely diffusible substance is involved. In addition the external location of the tongue graft should permit the injection of drugs into it which might indicate the nature of the receptors involved in bud formation. The finding that taste buds developed under the influence of intrinsic nerve fibers of the eye and that cuboidal epithelium of salivary ducts can give use to taste buds challenges the notion that only neurons in specific cranial ganglia and epithelia located in specific tongue regions can give rise to taste buds (Oakley, Brain Res. 75: 85, 1974). Of importance is the fact that by using the exteriorized tongue graft we found that there were two types of nerve fibers which could induce taste buds (i.e., one type fiber stained intensely for cholinesterase activity while the other did not). Further study showed that these same differences in nerve fiber cholinesterase activity was present in normal buds on the front (i.e., lacked cholinesterase activity) or back (i.e., had cholinesterase activity) of the tongue. This

difference in cholinesterase activity could be used to substantiate Oakley's proposition that tongue tissue regulates the type nerve it allows to enter and form buds (Oakley, J. Physiol, 188: 353, 1967). Taste responses differ in the front and back of the tongue and interestingly, Oakley found the responses did not change after crossing the taste nerves. It is possible to perform this cross-innervation experiment in the eye (combine nodose ganglion with grafts from the front of the tongue and combine the geniculate ganglion with the posterior tongue's vallate papilla) and see whether a particular tongue region excludes nerve fibers of a given cholinesterase type.

Proposed Course of Project:

A) Immunological studies:

- 1) Determine the immunological response to CNS homografts. I assume rejection will occur because it is known CNS tissue has histocompatibility antigens but tolerance to CNS tissue probably can be achieved.
- 2) Determine the immunological response to homografts in other species. All my previous studies were done in rats and confirmatory results in other animals is desirable.
- 3) Determine whether reflex connections can be established between the homografted neurons and the recipient neurons.
- 4) Investigate the use of the "nude mouse" (an animal with thymus agenesis and no cell mediated immunity) as an "in vivo" recipient for neuronal heterografts. For example, it should be possible to combine a rat ganglia with a mouse tongue graft or a newt ganglia with a rat tongue graft in the eye of the nude mouse to see if the trophic agent for taste buds is the same in various animals. If successful human tissue could be similarly studied (Dr. Goshgarian is presently doing this study).
- 5) Investigate whether mammalian neurons can perform trophic functions in the newt. For example, can rat sensory neurons induce limb regeneration in the newt. If they can, one must determine why no such regeneration occurs in mammals (study under supervision of Dr. Goshgarian).

B) Nerve Regeneration:

- 1) Determine whether the spinal cord environment is conducive to nerve fiber regeneration over great distances. A peripheral nerve will be implanted into a transected spinal cord and the distance it grows determined by horseradish peroxidase tracing (Dr. Goshgarian has been doing spinal cord transections and has perfected the HRP tracing technique).

- 2) Evaluate the effect of thyroid hormone in promoting nerve regeneration as reported by Kiernan (Exp. Neurol. 48: 88, 1975). A simple evaluation could be done by crushing the nerve to taste buds and comparing the rate of return of buds and their numbers in thyroid treated and non-treated animals. If thyroid hormone aids nerve regeneration it might be used to promote the growth of homografted neurons or of injured CNS tissue.
 - 3) Evaluate the effect of mucolytic and proteolytic enzymes on CNS regeneration which Mortinian and Windle (Anat. Rec. 181: 423, 1975) claim restores CNS function in injured spinal cord.
 - 4) Investigate the factors which allow the newt to naturally repair its injured spinal cord and see if they can be applied to the injured mammalian cord (studies being conducted by Dr. Goshgarian).
- C) Neuron-taste bud interactions:
- 1) Determine whether taste buds form when guinea pig ganglia and tongue grafts are placed in the eye. My rat studies need confirmation and my immunological studies will be extended to guinea pigs.
 - 2) Perform cross-regeneration studies with tongue grafts from the front and back of the tongue to see if tongue tissue regulates the type of taste nerve fiber that enters it.
 - 3) Perform electron microscopical studies to evaluate the morphological characteristics of taste buds and neurons in the eye.
 - 4) Determine whether nerve fibers (i.e., motor fibers) which do not induce buds actually enter the lingual epithelium or is some barrier preventing this. In situ reinnervation studies with the hypoglossal nerve will be performed.
 - 5) Test the specificities of epithelium and connective tissue in forming taste buds. Tongue and skin will be trypsinized to separate epithelium and connective tissue and then various recombinations made (i.e., skin epithelium and tongue connective tissue) to see if any of these specifically regulates the neural influence on bud formation.
 - 6) Determine whether drugs like plant lectins influence taste bud formation. Lectins bind to specific sugar molecules on cell membranes and if they bind to the correct molecule on epithelial

or nerve cell membrane they might inhibit taste bud formation. Such studies could provide insight into receptors involved in bud formation.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02077-03 LNC
PERIOD COVERED July 1, 1975 to June 30, 1976		
TITLE OF PROJECT (80 characters or less) Physiological and Biochemical Correlates of Cation Balance in Brain		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI; S. S. Goldman, Staff Fellow LNC NINCDS		
COOPERATING UNITS (if any) NONE		
LAB/BRANCH Laboratory of Neurochemistry		
SECTION Amino Acids & Electrolytes		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
SUMMARY OF WORK (200 words or less - underline keywords) It is the purpose of this study to resolve the <u>cold resistant</u> nature of the <u>brain of a mammalian hibernator</u> . The topics of interest and investigation are: (1) <u>Electrolyte metabolism</u> , primarily that associated with Na^+ and K^+ <u>balance</u> , <u>energy transduction</u> in biomembranes, (2) <u>membrane lipid turnover</u> , (3) <u>membrane biogenesis</u> . The use of a hibernating species of mammal, the <u>hamster</u> , is employed in these studies, since it can alter naturally when cold exposed, both the chemical and physical nature of its <u>neural elements</u> .		

Project Description:

Objective: To elucidate the biochemical basis of the regulation of cation balance in brain.

Methods: Cation balance in brain is considered to be the result of two categories of events: (1) anabolic processes, primarily active transport, which create a non-equilibrium distribution of electrolytes: (2) catabolic processes, such as membrane excitation and passive leaks, which permit electrolytes to redistribute toward equilibrium. These two categories can generally be distinguished on the basis of their responses to temperature: anabolic processes have high temperature coefficients while catabolic processes, as here defined, have a low temperature dependence.

The strategy employed in this research is to compare the electrolyte metabolism and related processes in warm-adapted hamsters with those of cold adapted (hibernating) hamsters. To this end a stock of hibernating hamsters is maintained.

Specific Methods: A working hypothesis of the current project is that alterations in membrane lipids are central to the cold-adaptation phenomenon. A physical approach was employed to investigate the fluid properties of the membrane. An apolar fluorochromic dye was incorporated into membrane fragments as well as their extracted lipids. Changes in the fluorescence polarization of the probe with respect to temperature were monitored and membrane viscosity was empirically determined.

Standard techniques of enzyme isolation and assay were used for further characterization of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ from brain of both the warm-adapted and hibernating hamster.

Major Finding: In the past year the fluid nature of brain membrane fragments and their associated lipids were described. It was found that the lipid adaptation in hibernation reported in Am. J. Physiol. 228: 834-838, 1975 does in fact favor a more fluid membrane at both normothermic and low temperatures. It was concluded from these studies that the fluid properties of the membrane of the adapted state are dependent upon the interaction of both cholesterol and membrane protein to the intrinsic phospholipid bilayer.

Studies on the kinetic behavior of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ from the adapted state has revealed the acclimation sites within the enzymatic cycle. These sites involve those steps within the catalytic cycle of the enzyme in which the major conformational events presumably occur. These conformational events are believed to represent in biochemical terms the sequential description of Na^+ and K^+ translocation across the membrane. Further these proposed acclimation sites are the temperature sensitive sites of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ in general.

One important finding from these kinetic studies arose from the effect of

very low temperature (7.5°C) on the enzyme from non-hibernating mammals. The enzyme becomes "locked" positionally in the membrane and possesses little $(\text{Na}^{+} + \text{K}^{+})\text{ATPase}$ activity whereas $\text{K}^{+}\text{NPPase}$ activity predominates.

Significance: The significance of the past year's work is useful in regard to understanding the energy transduction processes that occur in the membrane and how the lipid environment modulates these processes. One of the major energy transformations that occur in the membrane is the enzymatic system that accounts for electrolyte metabolism. The enzyme, $(\text{Na}^{+} + \text{K}^{+})\text{-ATPase}$, requires membrane phospholipids and further the physical state of these membrane lipids modulates enzymatic activity. As the membrane becomes less fluid or more viscid as a result of hypothermia, cation transport begins to fail. The failure is not the result of insufficient energy (ATP) to drive the system, but is the result of a magnification of the energy barriers that the enzyme must overcome. The energy barrier apparently is associated with an increased ordered state within the membrane bilayer. The use of temperature as a probe to resolve this enzymatic transduction mechanism and the use of the hibernator as a tool has given us an unique opportunity to assess the limitations of the hypothermic state, especially when employed in certain surgical procedures.

Further, the maintenance of a "dynamically" fluid membrane at normothermic temperature may help to explain the mechanism of action of a number of neuropharmaceuticals, neurotransmitter effects on membrane, and a number of neuropathologies that affect electrolyte imbalance in brain as a result of membrane lipid alterations. Localized ordering or disordering of the lipid bilayer as a result of these effectors and/or pathologies will modify electrolyte metabolism (both active and passive) in neural elements. Again the use of the hibernator has given us a potential to understand the dynamic state that the membrane possesses and its exploitation (i.e. the use of the hibernator) must be considered to the fullest.

Proposed Course: The lipid adaptation in brain that occurs as a result of cold exposure in those mammals that are destined to hibernate is the major thesis for forthcoming investigations. Two specific lines of approach are foreseen to address the problem associated with the biochemical basis of this unique mammalian adaptation. The biochemical events that describe the adaptation are at present unknown and deserve attention. Investigations are proposed on fatty acid desaturase, cholesterol turnover, and phospholipid biosynthesis in brain of both the warm-adapted and hibernating hamster. Of these three goals little is known about fatty acid desaturase in adult mammalian brain in general and nothing is known of these three areas in a hibernator.

Since the adaptation apparently involves both myelin sheath and neural membrane lipids, it is conceivable that the adaptation encompasses a restructuring process of both nerve cell membrane and myelin sheath lipid. An understanding of these events would be of paramount importance in elucidating those events associated with the turnover of neuronal membranes in general. The use of the hibernator as a tool may be instrumental and

offer a unique advantage to approach these questions.

The long range endeavor of this project will be an attempt to resolve the mechanism that describes and initiates the events associated with the lipid adaptation. The "trigger" may be either humoral or genetic. If genetic, a genomic sequence that is specifically encoded may only be expressed when the animal is exposed to the cold, thus an internal event. If humoral, a chemical message may be released by some element in the CNS that initiates the lipid metabolic events via an external route. Which ever process initiates and directs the adaptation its resolution and description may be instrumental in understanding the ontogenetic processes that occur in brain during development.

Publications:

1. Goldman, S. S. and Albers, R. W.: Cold Resistance of the Brain During Hibernation. Temperature Sensitivity of the Partial Reactions of the Na, K-ATPase. Arch. Biochem. Biophys. 169: 540-544, 1975.
2. Tower, D. B., Goldman, S. S. and Young, O. M.: Oxygen Consumption by Frozen and Thawed Cerebrocortical Slices from Warm-adapted or Hibernating Hamsters: The Protective Effects of Hybernation. J. Neurochem. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02215-01 LNC
PERIOD COVERED <p style="text-align: center;">July 1, 1975 through June 30, 1976</p>		
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Localization of ($\text{Na}^+ + \text{K}^+$)-ATPase in the Nervous Systems</p>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <div style="display: flex; justify-content: space-between;"> <div> PI: R. W. Albers, Section Chief, Lab. of Neurochem. OTHER: D. H. Jean, Visiting Associate </div> <div style="text-align: right;"> LNC NINCDS LNC NINCDS </div> </div>		
COOPERATING UNITS (if any) J. G. Wood, Department of Anatomy, University of Tennessee, Memphis, TN. B. J. McLaughlin, Department of Anatomy, University of Tennessee, Memphis, TN.		
LAB/BRANCH <p style="text-align: center;">Laboratory of Neurochemistry</p>		
SECTION <p style="text-align: center;">Enzyme Chemistry</p>		
INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</p>		
TOTAL MANYEARS: <p style="text-align: center;">0.6</p>	PROFESSIONAL: <p style="text-align: center;">0.5</p>	OTHER: <p style="text-align: center;">0.1</p>
SUMMARY OF WORK (200 words or less - underline keywords) <p> Antibody against purified ($\text{Na}^+ + \text{K}^+$)-ATPase of Electrophorus electric organ has been used to localize the active sodium transport system in brain of a smaller closely-related species at the ultrastructural level. The indirect peroxidase technique was employed. Localization to plasma membranes of neurons and glia was observed. In oligodendroglia the distribution extends to myelinated axons where it is restricted to the outermost edge of compacted myelin. Along axolemma of myelinated axons it is restricted to the portions at the node of Ranvier not covered by myelin. </p>		

Project Description:

Objectives: (a) To determine the distribution of $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ in structures of the nervous system; and (b) to explore the relationship of the observed ATPase distribution to the function of the nervous system.

Methods: Immunocytochemical localization by the indirect peroxidase method and subsequent examination by electron microscopy. Antibody to $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ is produced in rabbits. The adsorbed antibody is identified by its reaction with peroxidase-conjugate goat-anti-rabbit Fab.

Major Findings: Antibody raised against purified $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ of Electrophorus electric organ cross reacts with the $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ prepared from brains of the closely related small electric fish, Sternarchus albifrons. This species is cheaper, more readily available and more convenient to prepare for electron microscopy than Electrophorus. Our initial electron microscopic studies have shown excellent localization of cytochemical reaction product in animals treated with the antisera and essentially no reaction product in brains of animals treated with pre-immune sera.

Fine structural localization of the enzyme includes plasma membranes of the somata and dendrites of neurons, and the somata and processes of glia.

The reaction product associated with plasma membranes of oligodendroglia extends to myelinated axons where it is restricted to the outermost edge of the compacted myelin. The distribution along axolemma of myelinated axons is restricted to the portions at the node of Ranvier not covered by myelin.

Thus it is evident that the axolemmal proteins in the nodal region are not subject to free lateral diffusion.

Significance: The technique of immunohistochemical localization at the ultrastructural level is perhaps the most versatile and powerful tool that is presently available to bridge between the studies of molecular and cellular structures. Techniques of protein chemistry and enzyme kinetics permit one to make deductions about molecular structure and molecular events. Conventional electron microscopy has attained a level of resolution which readily visualizes macromolecular structures. Immunohistochemistry offers a general approach to the identification of these structures in terms of their biochemical functions. In the case of membrane proteins, many biochemical and physical measurements are limited to the soluble forms of these proteins. The combination of biochemical and electron microscopic techniques provides a means of determining higher orders of molecular organization that may be destroyed by solubilization procedures.

The information about localization within the membrane raises basic questions such as how such proteins are "anchored" within a particular segment of the membrane. As the normal pattern of localization is established, this will provide a basis for comparison with pathological states which involve

defective membrane functions.

Future Course: Several observations concerning the localization of the $(Na^+ + K^+)$ -ATPase in relation to synaptic and intra-cellular membranes will require further studies. These will probably include studies of sub-cellular fractions. They will also involve denervation, regeneration and the use of various pharmacological blocking agents.

We plan to extend the technique to other species for particular purposes. Thus we wish to investigate the ontogeny of sodium pump localization. This can be best done in chick and will require purification and development of antisera to chicken brain $(Na^+ + K^+)$ -ATPase.

Preliminary experiments in collaboration with Dr. T. H. Oh of the Department of Anatomy, University of Maryland, have shown that explants of spinal cord of Electrophorus can be maintained and cell outgrowth occurs. Cultures of electric organ have been maintained but without appreciable outgrowth.

We plan to investigate possible neurotropic effects on electroplax development. Dr. Oh has been successful in delineating specific neurotropic protein factors that stimulate growth and development of chick and mammalian skeletal muscle. Electric organ tissue is a skeletal muscle derivative. Because of its high degree of specialization in terms of cholinergic membrane receptors and sodium pumping, we consider that the development of a system to culture this tissue would open up numerous possibilities for the study of factors which influence the differentiation and biosynthesis of excitable membrane components.

Publications: None.

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PERIOD COVERED <p style="text-align: center;">July 1, 1975 through June 30, 1976</p>								
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Biochemical Regulation in Astrocytes</p>								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 70%;">R. W. Albers, Section Chief, Lab. of Neurochemistry</td> <td style="width: 20%;">LNC NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>D. H. Jean, Visiting Associate</td> <td>LNC NINCDS</td> </tr> </table>			PI:	R. W. Albers, Section Chief, Lab. of Neurochemistry	LNC NINCDS	OTHER:	D. H. Jean, Visiting Associate	LNC NINCDS
PI:	R. W. Albers, Section Chief, Lab. of Neurochemistry	LNC NINCDS						
OTHER:	D. H. Jean, Visiting Associate	LNC NINCDS						
COOPERATING UNITS (if any) <p style="text-align: center;">NONE</p>								
LAB/BRANCH <p style="text-align: center;">Laboratory of Neurochemistry</p>								
SECTION <p style="text-align: center;">Enzyme Chemistry</p>								
INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</p>								
TOTAL MANYEARS: <p style="text-align: center;">0.4</p>	PROFESSIONAL: <p style="text-align: center;">0.2</p>	OTHER: <p style="text-align: center;">0.2</p>						
SUMMARY OF WORK (200 words or less - underline keywords) <p style="text-align: center;">This project has been terminated.</p>								

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01303-11 LNC
PERIOD COVERED July 1, 1975 through June 30, 1976		
TITLE OF PROJECT (80 characters or less) Elucidation of factors regulating the metabolic and functional characteristics of skeletal muscle sensori - motor components.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Herbert Yellin LNC NINCDS		
COOPERATING UNITS (if any) NONE		
LAB/BRANCH Laboratory of Neurochemistry		
SECTION Neural Development & Regeneration		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 1.1	PROFESSIONAL: 1.0	OTHER: 0.1
SUMMARY OF WORK (200 words or less - underline keywords) This project has been terminated.		

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